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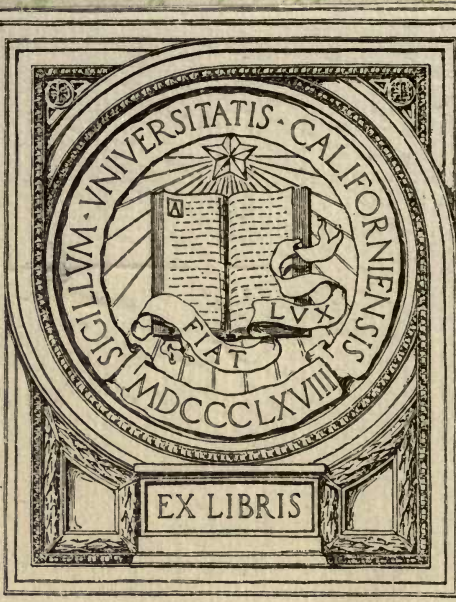
LABORATORY METHODS OF HISTOLOGY AND BACTERIOLOGY

HOLMAN



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LABORATORY METHODS

OF

HISTOLOGY AND BACTERIOLOGY.

BY

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NASHVILLE, TENN.:
NATIONAL BAPTIST PUBLISHING BOARD.
1903.

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PREFACE.

This manual of Histology and Bacteriology has been more particularly prepared to facilitate the study of these important branches of medicine by the author's classes. While the author makes no pretension to original research in this book, he has endeavored to include the useful and practical points of the more exhaustive works on the subject. In every case the shortest and best methods are used. He has avoided technical terms where practicable and has sought to express the truth in simple language. He has provided blank sheets for drawing and extra notes before and after each part of this volume.

The author wishes to express his gratitude to friends for encouragement, especially to Dr. G. W. Hubbard, M. D., Dean of Meharry Medical College, and Prof. W. Patterson, A. M., Assistant Professor of Chemistry in the same institution. He wishes to thank his assistant, Mr. A. Lyman Paey, for the illustrations, most of which being original, having been sketched from actual specimens prepared by the students in the laboratory of Meharry Medical College. He also

wishes to express his gratitude to the National Baptist Publishing Board for the excellent and artistic manner in reproducing the illustrations and getting out the work.

The author embraces this opportunity to acknowledge his obligations to the following authorities for information, viz.: Sobotta's Human Histology, Bohm and Davidoff's Histology, Leroy's Essentials of Histology, and McFarland, Eyre and Sternberg's Bacteriologies.

J. H. HOLMAN.

Meharry Medical College, Nashville, Tenn., May, 1903.

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PART I.

HISTOLOGY

LABORATORY METHODS OF HISTOLOGY AND BACTERIOLOGY.

CHAPTER I.

THE MICROSCOPE AND ITS ACCESSORIES.

The microscope is an instrument which magnifies an object by means of its optic apparatus. There are two kinds of microscopes, the simple and compound. The compound is used in Histology and Bacteriology. The parts and use of the same will be taught from time to time in the Laboratory. The base is the part that rests upon the table and supports all the rest of the instrument. The pillar is the perpendicular part extending from the base to the back part of the stage. The arm is the part that is attached to the pillar and works on a hinge-joint. The mirror is attached to the arm in front and works by means of a universal joint; its function is to give light to the observer while examining the object. The stage is the platform upon which the object rests while it is being ex-

amined. The aperture is the circular opening in the stage; its diameter can be varied according to the amount of light required to see the object distinctly by the use of the diaphragm, which is the circular disk placed under and attached to the platform. The body is the cylindrical attachment attached to the arm in front and supporting the coarse adjustment, draw tube and objectives. The eyepiece and the objectives are the main parts of the instrument. Since the eyepiece and objectives are the essential parts of the instrument, it is very important that we know enough about them to use them intelligently. The eyepiece consists of a field glass and an eyeglass, which are placed in the positions indicated by their names. The coarse adjustment is placed on the rear of the arm, at the point where the arm and body meet. Its use is to obtain a focus. The fine adjustment is placed on the top of the arm and is used to complete the focus obtained by the use of the coarse adjustment. The draw tube is placed in the body of the instrument and is used to increase its magnifying power.

Exercise No. 1.—Study of the Microscope.

Make a study of the microscope at your locker and learn to use all the parts. Take the material given you and find a focus of the same. Your focus is the point

where you can see the object best. Since you understand the principles of the microscope, we will not spend the time to explain them, but will use the instrument.

CHAPTER II.

MANIPULATION AND CARE OF THE MICROSCOPE.

1. In taking an instrument from the case, grasp it by the base and pillar.

2. Free it from dust, with the paper supplied.

3. Place the instrument on the worktable, with the opening of the base from you.

4. Place your eye over the eyepiece, and at the same time manipulate the reflector until you get a good light.

5. Place the object to be examined on the stage and lower the objective to a point just above the slide and cover-glass, with your head on a level with the stage while doing so.

6. Now regulate the light by means of the diaphragm.

7. Place your eye over the eyepiece and rack upwards until you reach the point where you can see the object.

8. Note what you see in your notebook.

9. Make drawings of what you see.

Handle the instrument with more than ordinary care; never let anything touch the lenses. The instrument must be kept clean. Be careful and learn to use the instrument with both eyes open. Do not use it so long that an aching sensation will be produced in the eyes.

The accessories to the microscope are many, but we will not use all of them; since we want a working knowledge of the following we will learn their use:

The Substage, which is placed under the stage, is to support the Abbe condenser.

The Iris diaphragm is used to regulate the light which passes through the condenser.

The Abbe is a lens of a very short focal distance and gives a very strong light.

The Mechanical Stage is an instrument used to manipulate the object while upon the stage.

The Camera Lucida is an instrument used to assist the student in making the drawings.

The Polariscope is used to examine objects by means of polarized light.

The Micrometer is an instrument used to determine the magnifying power of the microscope. There are two kinds of micrometers, viz.: an eyepiece and a stage micrometer. Determine the magnifying power of the instrument at your desk.

1. Take the stage micrometer and place it upon the stage of the microscope.

2. Build a platform as high as the stage of the instrument, using books for the purpose.

3. Place upon your platform a piece of white paper.

4. Now focus the lines on the stage micrometer, keeping both eyes open.

5. The lines on the micrometer will be seen upon the paper.

6. Now make a pinhole in the paper between any two lines.

7. Multiply the distance between the pinholes in the paper by the denominator of the fraction on the scale of the micrometer. The product will be the magnifying power of the instrument.

The Microtome is an instrument that is used to cut tissue into very thin sections.

The Paraffine Bath is an apparatus that is used to infiltrate the tissue with paraffine.

The Cornet Forceps is an apparatus used to hold the cover glass.

The Centrifuge is an apparatus used to precipitate the solid from a fluid.

Slides and Cover-Glasses are accessories used to retain or protect the tissue (or object).

Exercise No. 2—Use the Polariscope.

1. Place the microscope upon the table and take off the objective.

2. Attach the analyzer.

3. Reattach the objective to the lower end of the analyzer.

4. Place upon the stage the specimen.

5. Now place upon the specimen the selenite plate.

6. Now attach the polarizer and proceed to examine the preparation.

Use the Abbe:

1. Place the object upon the stage and get a good light.

2. Screw the Abbe around in place and regulate the light by the use of the iris diaphragm.

3. Examine, note and draw.

Exercise No. 3.—Use the Microtome.

1. Place the tissue in the clamps of the instrument and fasten it.

2. Set your knife so that it will be at an angle with the tissue.

3. Have the block of tissue of the right size, and gently but firmly draw the knife across the tissue.

4. Regulate the thickness of the tissue by the use of the milled head on the under surface of the microtome. (B. & L. is the best for students' use.)

Exercise No. 4.—Use the Centrifuge.

1. Attach the instrument to the table and place the tubes in place.

2. Revolve the tubes moderately for three minutes.

3. Take the sediment out and examine with the microscope.

The Hæmatokrit is used in the same way, only revolved more rapidly.

Exercise No. 5.—Make a spread of yeast-plant.

1. Obtain some yeast and place it in a little water in a test tube; set aside in a warm place for a short while.

2. Take a pipette and compress the bulb so as to get some of the yeast and water in the pipette.

3. Now place a small drop of this material on the slide and examine.

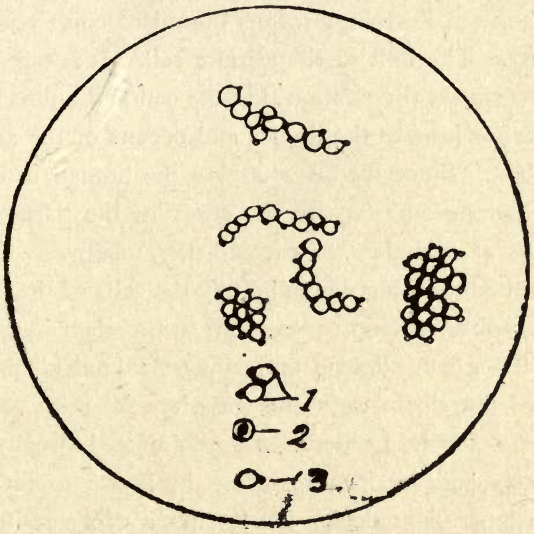
4. Examine with H. P. (1-6 in. Obj.)

5. Observe the morphology, grouping, budding and search for a chain.

6. Make drawings and note all you see.

This lesson is to illustrate the mode of reproduction of cells, morphology (or shape), the contents of cells,

the function, and the spores (or seed of cells). We use the plant cells because they are very large and are easy to obtain and possess all the properties of the ani-



CELLS OF BREWERS' YEAST.

1, A group of two cells; 2, dark spot represents a vacuole; cell forming a bud.

mal cells. The size of the yeast cells is 1-4000 to 1-2500 of an inch in diameter. Their form is ovoidal. They have the power to produce fermentation. In studying the plant under the microscope you will do well to note whether there is any chlorophyll in the plant or not. In this way you will be able to make your

experiments of very great value to you. This work is to introduce the student to the study of Histology. The word Technic means the various manipulations necessary to get the tissue ready for the microscope.

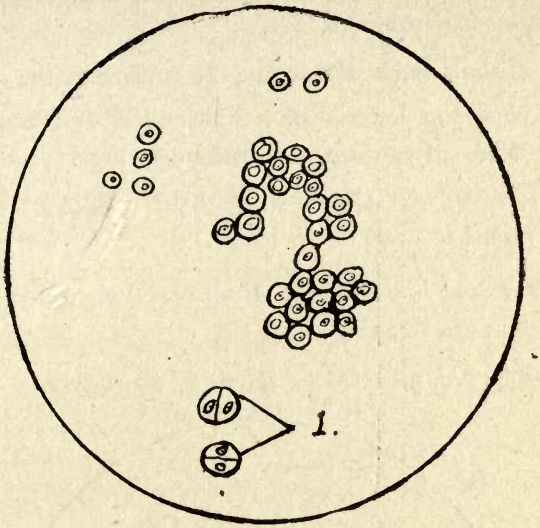
The term Histology means the microscopic study of tissues. The unit of tissues is a cell. It is our work to investigate the relation that the cell and intercellular substance bear to the tissues and organs of the animal or plant. Since we are studying the human body we will confine ourselves to the study of the tissues and organs of animals, *i. e.*, cat and dog, mostly.

In the beginning we will study the cells of the yeast-plant, spirogyra and protococcus in the plant kingdom, and the green euglena and slipper animalcule in the animal kingdom—each one for a special purpose: the euglena, for its property of containing chlorophyll in its body and for its possessing the power of motion; the slipper animalcule, for its mode of reproduction and its mode of locomotion.

We will begin with the yeast plant and go upwards and see if there are any changes in the vegetable kingdom that resemble those in the animal kingdom.

Exercise No. 6.—Make a spread of Protococcus.

1. From the green growth on a tree or fence, take a small bit of the growth and place it in a petri-dish, moisten and put in a warm place for twelve hours.



PROTOCOCCUS.

Showing morphology, grouping and fission.

2. When the cells have begun to vegetate place some of them on the slide and irrigate with water.

3. Cover with cover-glass and examine.

4. Use H. P. and observe grouping, cell contents, and reproduction.

5. Irrigate with acetic acid and search for a nucleus.

The lesson here is to find some reagent that will find a nucleus without staining the preparation. Acetic acid is the one. This is used in all of our work when we look for a nucleus.

Exercise No. 7.—Make a spread of scrapings of some organ and examine.

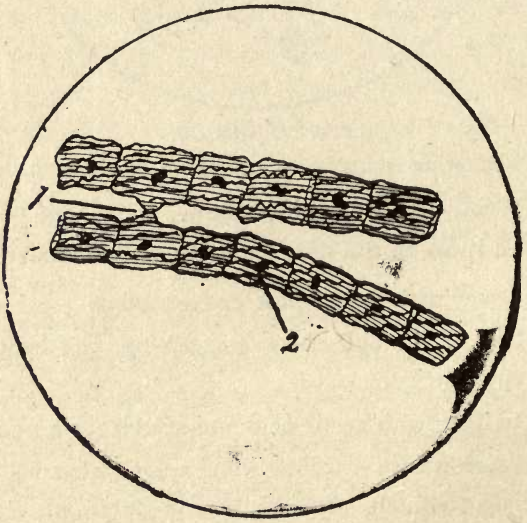
1. Take a knife and scrape the surface of the organ and place the material on a slide; cover and examine.

2. Note all you see, and make drawings.

Exercise No. 8.—Make a normal Salt Solution. (NaCl. 7 grams to 1,000 c.c. of water.)

This fluid is to be used in all cases when you want to dilute the scrapings.

Exercise No. 9.—Make a spread of Spirogyra.



SPIROGYRA.

1, Showing mode of reproduction; 2, nucleus.

1. Obtain from a pond or brook a small quantity of the growth and place it in a glass vessel with some water.

2. Examine with H. P.

3. Search for nucleus, note the shape of the cells, note the arrangement of the cells in the filament, observe the arrangement of the chlorophyll in the cells, irrigate with acetic and make drawings.

Exercise No. 10.—*Irrigation.*

1. Place the cover glass on the spread.

2. Place a drop of reagent on one side of the preparation and a piece of blotting paper on the other.

3. Look through the instrument.

4. Note what you see and draw.

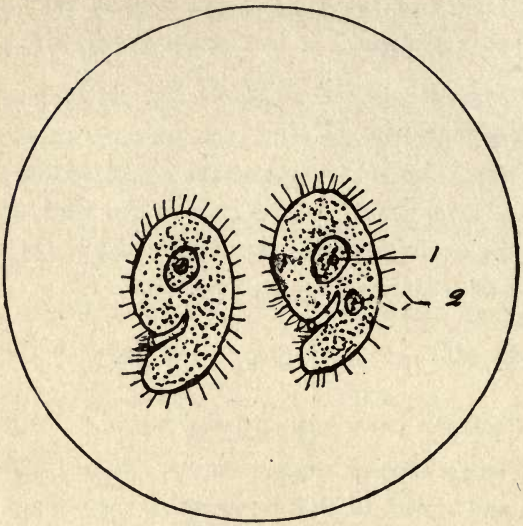
Any reagent may be used for irrigating. Irrigation is only used on fresh spreads.

Exercise No. 11.—*The Slipper Animalcule.*

1. Take some of the scum from the top of the hay infusion.

2. Examine with H. P.

3. Make out all the structures of the animal, note and draw.



SLIPPER ANIMALCULE.
1, Nucleus; 2, cilia.

Exercise No. 12.—*Dissociate some tendon of an ox.*

1. Place some of the tendon in enough weak soda solution to cover it and let it remain for two days.

Exercise No. 13.—*Tease some of the tendon which has been dissociated in the soda solution.*

1. Fix a sewing needle in a pen-holder.

2. Use as a teasing instrument.

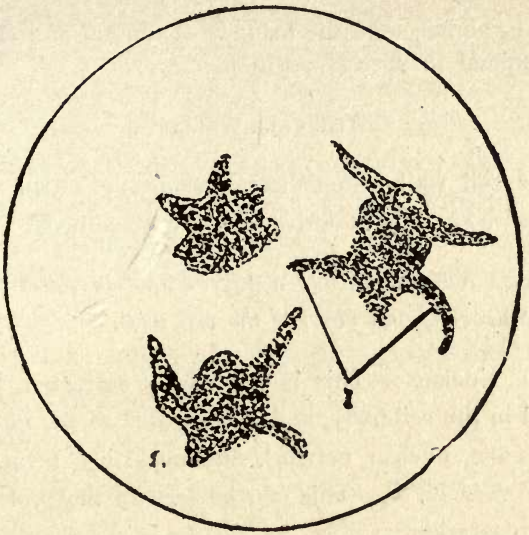
3. Tear the tissue into small bits and place them on the slide.

4. Examine with H. P. and L. P. When using the L. P. you may dispense with the use of the cover-glass.

CHAPTER III.

GENERAL HISTOLOGY.

The Cell:—A cell is the unit of all the living organisms. All cells originate from a pre-existing cell. A complete cell consists of the following: a cell wall, protoplasm, nucleus, nucleolus and centrosome. All cells are not complete: some may have a nucleus, and some may not; some may have a cell wall, and some may not. A complete cell is called a typical cell, and one that is incomplete is called an atypical cell. The cell body is a mass of protoplasm, having in it a varying amount of delicate fibers called "spongioplasm." Along the course of these we see several little nodes. These nodes are called microsomes. That part of the protoplasm near the nucleus is called the nucleoplasm, and that part near the cell wall is called the exoplasm. The power of motion is located in the protoplasm. This is illustrated in the Amoeba, a small animal which moves by means of pseudopods, *i. e.*, a part of the pro-



AMOEBA.
1, Pseudopods.

toplasm flows to one side of the body of the animal, and then the rest of the protoplasm flows in the same direction, until the whole animal changes its position.

Exercise No. 14.

1. From the scum of the hay infusion pick out a submerged leaf.

2. Apply the cover-glass to the leaf and place on the slide.

3. Examine, note the manner of motion and sketch the animal in several positions.

THE CELL WALL.

The cell wall is a delicate membrane surrounding the cell-body, and is derived from the cell-body.

Exercise No. 15.—*Make a microscopic preparation of Protococcus and observe the cell wall.*

The Nucleus:—This is the highly refractive body found in the cell-body, and is composed of the nuclear membrane, nuclear network and nucleolus, seen only under very H. P. This can be seen in the protococcus preparation.

Centrosomes:—These are highly refractive bodies seen in the nucleus, and they preside over the reproduction of cells, while the nucleus transmits the hereditary traits of the parent cell to the new one.—*Leroy.*

The properties of a cell are motion, reproduction, metabolism, growth, irritability and function. Metabolism is that property of a cell by which it takes in nutriment and assimilates the same, and the property of excreting the waste matter, which is the result of metabolism. Irritability is the property of a cell to respond to nervous stimulation. The function of a cell is the work the cell will do. Reproduction of a

cell is the power to reproduce a like cell. This is accomplished in two general modes: Direct and Indirect. The *Direct* is observed in the protococcus and yeast plants. The *Indirect* is studied in the higher animals. For convenience the stages of changes are noted separately. They are as follows: (1) Resting nucleus; (2) the Skein; (3) the Rosette; (4) the Aster; (5) the Diaster; (6) the Daughter Rosette; (7) the Daughter Skeins; (8) the Daughter Nuclei. Each one of these will be observed in the experiment with the growing tip of the onion.

Exercise No. 16.—

1. Grow some onions in a glass jar in some water.
2. Cut off some of the tips of the growing rootlets.
3. Fix them in formalin solution.
4. Harden in alcohol for 1 hour.
5. Dehydrate with absolute alcohol.
6. Place the tissue in equal parts of ether and absolute alcohol for twelve hours; then place it in thin celloidin for twelve hours.
7. Now place it in thick celloidin for twelve hours.
8. Embed, section, mount and stain.
9. Examine with H. P. and the oil immersion.

Technic for making the solution. The formalin is made as follows:

1. Take a 40 per cent solution of formaldehyd in water or alcohol. Make a 10 per cent solution. The celloidin solution is made by taking 5 grams of celloidin and 100 grams, *i. e., c.c.,* of the mixture of equal parts of ether and absolute alcohol. Mix.

2. The thick celloidin is made by taking the same mixture and adding celloidin until the mixture is as thick as a sirup.

By sectioning we mean cutting the tissue into very thin sections, so that they can be easily made transparent, by the next steps which are to follow. By clearing a tissue we mean that we make the tissue so transparent that it is of the same transparency as that of the glass slide on which the tissue is mounted. Several chemicals are used for this purpose. They all depend on their indices of refraction for their value. They are called clearing agents.

A piece of tissue to be carefully and properly mounted must go through the following steps:

1. The cells must be killed.
2. The cells must be fixed.
3. The tissue must be hardened.
4. The tissue must be dehydrated.
5. The tissue must be dried.
6. The tissue must be infiltrated.
7. The tissue must be embedded.

8. Sectioned; embeding material taken out; then the tissue fixed on the slide, stained, mounted and labeled. After the student has carefully done this he will not have any trouble.

Exercise No. 17.—

1. Apply to the slide a section of the outer skin of an onion-bulb. (Apply the section and add the stain.)

2. Wash in water to remove the excess of stain.

3. Dry with blotting paper; add a dehydrating medium.

4. Wipe off the dehydrating medium, which is usually glycerine.

5. Add xylol.

6. Apply balsam to the section and place cover-glass on the section balsam downward.

7. Label and keep in a horizontal position until balsam hardens (or study at once). Make out all you can about cells in this preparation; *i. e.*, shape, size, contents and motility.

Exercise No. 18.—

Make a preparation similar to that above and apply a drop of iodine solution and examine. Now remove the cover and apply a drop of sulphuric acid. The

stain will take reddish-brown; then the acid will attack the cellulose and convert it into dextrine; then the whole will be reddish-brown. At first all the starch will be blue, due to the presence of the iodine solution. Look for Brownian movement; this is an independent movement which is not due to vitality.

Exercise No. 19.—*Make a section of a potato and search for starch granules.*

1. Place the potato in the microtome and cut sections 20 microns thick.

2. Place a section on the slide and search for a good field in which to make observations.

3. Note the morphology of the cells and the contents of the same.

4. Place a cover on the section and irrigate with iodine solution.

5. Make notes.

This is the mode of making a micro-chemical examination. Note that the morphology in this case is not the same as in the onion cell.

CHAPTER IV.

THE DEVELOPMENT OF ANIMAL TISSUE.

1. The cell or ovum of the female, which is generated in the ovary, passes down the fallopian tube and meets the spermatozoön, the male element, provided conception has occurred.

2. The ovum there multiplies by indirect division into a mass that resembles a mulberry; hence the mulberry stage.

3. The cells do not all grow with the same rapidity, so that there are soon formed three distinct layers of the original mass, *i. e.,* the *epiblast,* the *hypoblast* and the *mesoblast.* The *epiblast* is the uppermost layer, the *hypoblast* is the under layer, and the *mesoblast* is the middle layer.

Now is the time when tissue differentiation begins. All the tissues of the body are formed from these three layers; hence the terms: mesoblastic, hypoblastic and epiblastic, so often used in medical literature. A tissue is composed of cells and intercellular substance, working in common to accomplish some purpose.

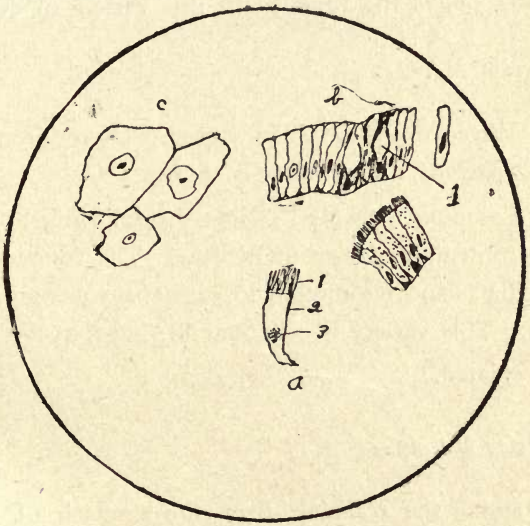
In this manual you will find the tissue origin under each tissue, as well as on this page. From the epiblast or ectoderm are derived the following tissues: nervous tissue, the epithelium, covering the surfaces of the body, the enamel, the nails, the hair, the organs of special sense, and all glands, except those which open into the alimentary tract, from the œsophagus downward; from the mesoblast or mesoderm are derived blood, blood vessels, all the connective tissues, muscles, dentine and cementum; from the hypoblast or hypoderm are derived the epithelial lining of the alimentary canal and the glands opening into it. Tissues may be placed into four groups: *epithelial, muscular, nervous and connective.*

CHAPTER V.

EPITHELIAL TISSUES.

In this manual Epithelial Tissue is considered first.

Exercise No. 20.—



EPITHELIAL CELLS.

a, Squamous; b, columnar; 1, goblet cells; a, ciliated epithelial; 1, cilia; 2, cell wall; 3, nucleus located in the protoplasm.

Collect upon the tongue some saliva and place it on the slide; cover and examine with H. P. Do you see any blood vessels? Are the cells all of the same shape? What figure would be the nearest to their shape? What have you concluded as to the manner in which they get their nourishment? Do they resemble scales? If so, they are of the squamous variety. There are four varieties and it is our purpose to study them in detail. These cells are reproduced by mitosis. Give the function of this variety of cells.

Exercise No. 21.—

1. Place upon the slide some scrapings from the upper part of the pharynx of a frog.

2. Examine with H. P. Note the following: shape, cilia, contents and size of nucleus. The functions of the cilia is to give motion to substances passing over them. This variety is columnar in shape, at the same time ciliated.

Exercise No. 22.—

Examine the scraping from the stomach of some animal and note the shape of the cells there. This is done in the same manner as the others. You will find that the cells are of a columnar variety.

Exercise No. 23.—Make a preparation of liver cells

Do the same as Exercise No. 21.

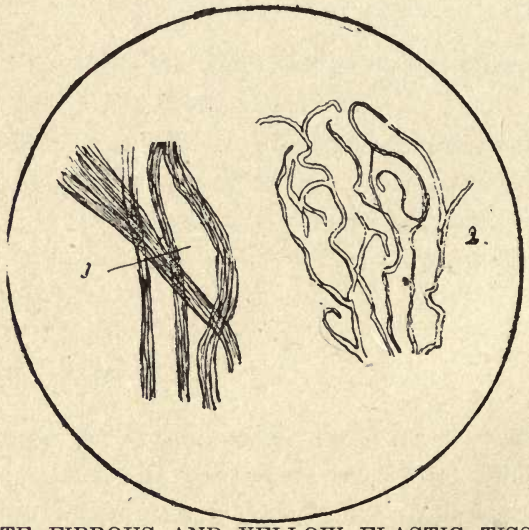
P. S.—A scalpel is used in scraping.

Under the head of Epithelial there are several modified cells. Some of them contain mucin and others a pigment. Sometimes their shape takes on the nature of all the four varieties. They are then called *transitional epithelial cells.* Again they are modified according to function. They are then called *specialized epithelial cells; i. e., glandular* and *neuro epithelial.*

CHAPTER VI.

CONNECTIVE TISSUE.

Connective Tissue is of mesodermic origin. It serves to hold the different parts of the body together, and contains more intercellular substance than the epithelial tissue. It serves to support the organs of the body as well. There are ten varieties of this tissue:



WHITE FIBROUS AND YELLOW ELASTIC TISSUES.
1, White fibrous; 2, yellow elastic.

I.—WHITE FIBROUS TISSUE.

Study the white fibrous tissue.

1. Tease a piece of tendon of an animal and place it on the slide.

2. Stain with carmine.

3. Observe the fibers running in a wavy line across the field. This tissue is found in the following places: tendon, omentum, subcutaneous tissue and a few other places.

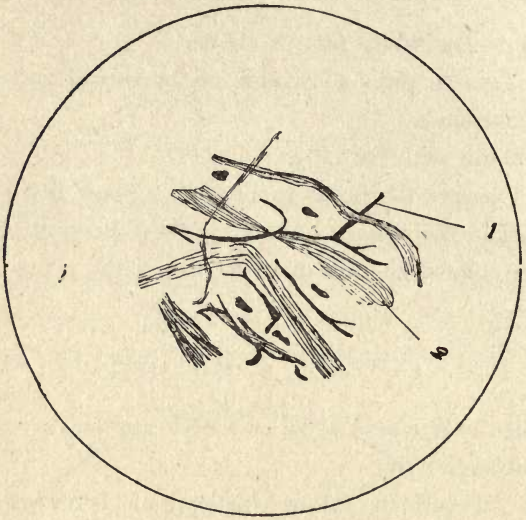
II.—YELLOW ELASTIC TISSUE.

Take the ligament of an ox's neck and prepare, stain, mount and study.

To differentiate yellow elastic tissue from white fibrous: take a scalpel and cut each one. The white fibrous will remain unaltered, while the yellow will curl up. Under the microscope the white will appear to be in a straight or wavy line, while the yellow will appear to be in branched processes. If you boil them the white will yield gelatin, and the yellow yields elastin.

III.—AREOLA TISSUE.

It is composed of white, fibrous, yellow elastic and adipose tissues, in varying amounts.



AREOLA TISSUE.

1, Yellow elastic tissue; 2, white fibrous tissue. The dark spots represent wandering cells.

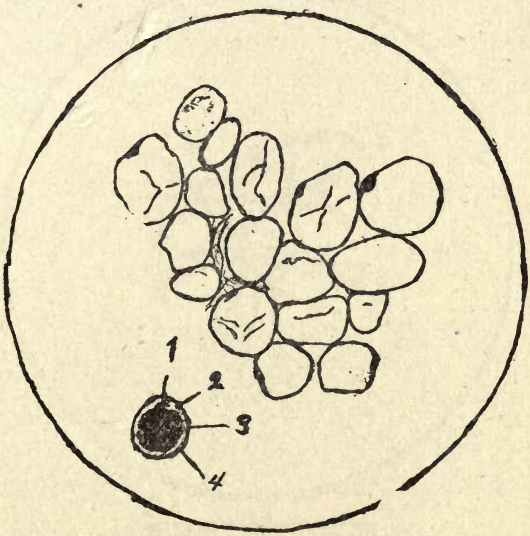
IV.—MUCOUS TISSUE.

This tissue is composed of large branching cells surrounded by a soft gelatinous substance (mucin). In embryo it is found in the umbilical cord. Study this tissue.

Technic: Make a spread of some fluid that exudes from the cut end of an umbilical cord and observe the shape, size and contents of the cells.

V.—ADIPOSE TISSUE.

This tissue is composed of large, poorly stained cells, and is found in a large number of places in the body. These cells are bound together by areola tissue. The fat cells are derived from the fixed connective cells.



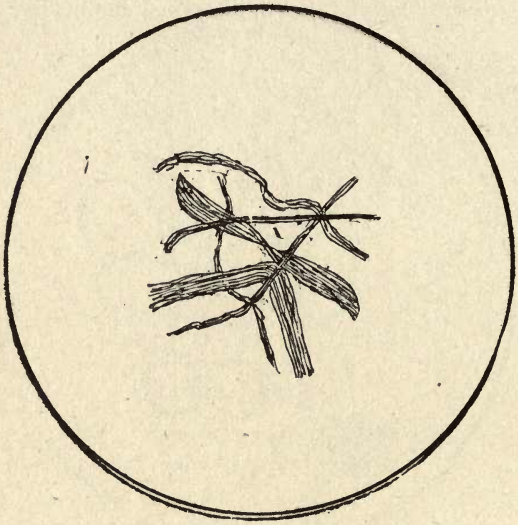
ADIPOSE TISSUE.

1, Nucleus; 2, protoplasm; 3, fat drop; 4, cell wall.

The protoplasm is converted into fat, and the nucleus is pushed to one side and finally leaves the cell-body. Fat surrounds every tissue of the body except the brain and spinal cord. This tissue is used as a cushion for the eye.

VI.—RETIFORM TISSUE.

This variety of tissue is composed of a number of white fibers and a varying amount of connective tissue cells.



RETIFORM TISSUE.

Showing only the fibrous tissue.

Exercise No. 24.—

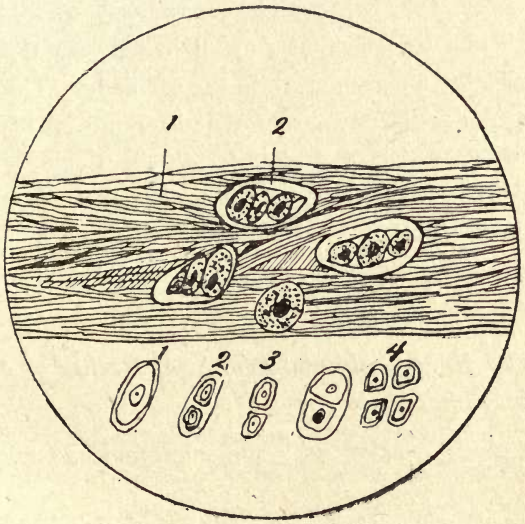
Take a section of a fresh spleen and shake it in an indifferent fluid for several minutes; then place it on the slide and examine. An indifferent fluid is a fluid that has no effect on the tissue which is placed in it.

VII.—ADENOID TISSUE.

This tissue is simply retiform tissue having a varying amount of lymphoid cells entangled in its meshes.

VIII.—CARTILAGE TISSUE.

This is a variety of connective tissue that is widely distributed. There are three main varieties of cartilage; viz.:



WHITE FIBRO-CARTILAGE.

1, White fibers; 2, cartilage cells; 1, 2, 3, 4, cartilage cells in different stages of reproduction.

White Fibro-Cartilage.—This variety is composed of the following material: matrix, capsule, perichondrium, cells and lacunæ. The matrix has embedded in it a few white fibers: hence the name. The matrix is a homogeneous material, semisolid in consistency, and will yield chondrin on boiling. The oval shaped cells lie embedded all in the matrix, in groups of two, four and eight, etc. The cells are those oval bodies scattered all in the matrix. The lacunæ are those hardened areas found near the cells on all sides, those white threads that run all in between the cells and give the fibrous nature to the cartilage. The perichondrium is the membrane that is found on the outside of the cartilage, and is composed of two layers: one to protect the cartilage; the other to furnish new cells in the growing cartilage.

Hyaline Cartilage is composed of a matrix, perichondrium, cells and lacunae.

Exercise No. 25.—Mount a T. S. of trachea of a cat; stain with carmine.

1. Cut the section with the microtome 20 microns thick.

2. Place the section on the slide and stain.

3. Wash off excess of stain with acid alcohol.

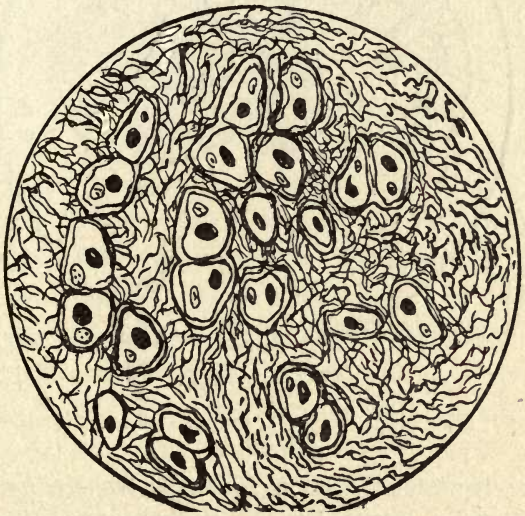
4. Wash in 70 per cent. alcohol; then 80 per cent. and 90 per cent.

5. Wash in 100 per cent.

6. Clear up with some of the clearing agents.

7. Dry and mount in balsam.

T. S. is the abbreviation for transverse section.

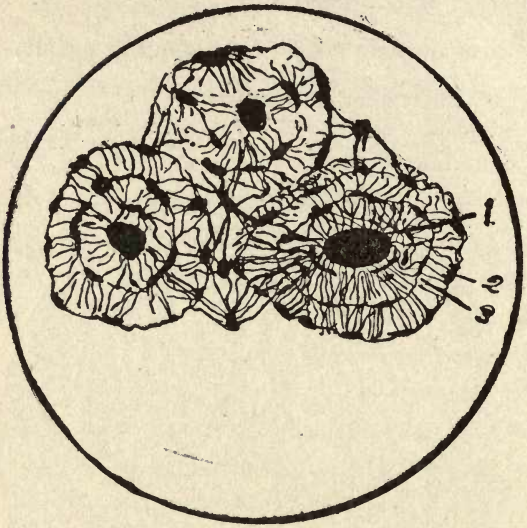


FIBRO ELASTIC CARTILAGE.

The Fibro Elastic Cartilage.—This variety of tissue is composed of yellow elastic tissue in addition to the material in the white fibrous cartilage.

IX —BONE TISSUE.

This variety of tissue gives shape to the body and is therefore very hard. It is composed of the following



T. S. BONE.

1, Haversian canal; 2, lacunae; 3, canaliculi.

parts: *Haversian system, Haversian canals, lamellae, lacunae, canaliculi* and *bone cells.* The Haversian system is composed of Haversian canals, a number of canaliculi, a number of lamellae and a number of bone cells. The cells lie in the bone matrix in much the same way as they do in the cartilage matrix. The lacunae are also very much the same. The bone cells send out processes for the purpose of conveying nutriment to all parts of the bone; these processes are called canaliculi. The Haversian canals carry the small

blood vessels into the bones. The lamellae, arranged around the Haversian canal, are called the Haversian lamellae. The lamellae running parallel with the outer surface of the bone are called the ground lamellae, and those running between the Haversian system, are called the interstitial lamellae. There are little canals running from the periosteum to the nearest Haversian lamellae. These canals are called Volkmann's canals. Occasionally uncalcified lines are seen passing obliquely or transversely through the lamellae. These are called the lines of Sharpey. The periosteum consists of fibrous connective tissue, and is divided into two layers. One layer is the bone-forming layer and the other is the bone-protecting layer. Bone-marrow is composed of two kinds of cells, the red and yellow. The yellow is mostly of the fat variety, and the red is mostly of red blood cells. The blood vessels of the bone tissue are distributed through the Haversian system and the canals of Volkmann. The periosteum is richly supplied with blood vessels.

Bone develops in two ways: centers of ossification and perichondrial. The perichondrial is as follows: the cells of the immature bone pass down the lines of Volkmann and locate themselves in a center called a center of ossification. Ossification means the depo-

sition of salts of lime in the tissues. These salts are usually deposited in cartilage, and then it is called bone. Dentine is a variety of bone, and will be discussed more fully when we come to the subject of the teeth.

Exercise No. 26.—Make a preparation of cartilage from the end of bone in the fresh state.

1. Take a sharp knife and cut a piece of cartilage from the end of a long bone and section.

2. Mount in salt solution.

3. Stain.

4. Study, draw and note.

Look for the following: matrix, cells, capsule, lacunae and perichondrium.

Exercise No. 27.—Examine the L. S. of bone given you.

Look for the following: Haversian system, Haversian canal, lacunae, bone cell and canaliculi.

Exercise No. 28.—Examine the T. S. section of the bone given you.

Look for the same as you did in the previous exercise. Make drawings in both cases; note and study.

Exercise No. 29.—Examine the cuts in your text and make a drawing of all you see.

CHAPTER VII.

BLOOD TISSUE.

[The tenth variety of Connective Tissue mentioned in this work.]

Blood is a tissue of mesodermic origin, and has a fluid intercellular substance, known as plasma. The cellular elements are many, viz.: white *corpuscles, red cells,* and the *platelets.* The blood is distributed to all parts of the body by means of tubes called arteries, and is returned to the heart by means of the veins. The capillaries are the terminals of the arteries and the beginning of the veins. Their walls are very thin, and admit a change of gases between the blood and the tissues.

Exercise No. 30.—*Obtain a drop of blood from your finger.*

1. Sterilize the lance and prick the part selected.

2. Wipe off the first drop of blood with a clean cloth wet in alcohol.

3. Constrict the part above the puncture and obtain another drop.

4. Place this drop on the slide and cover, or touch the edge of two clean cover-glasses to the drop, so that a very thin spread of blood can be secured between them; place on the slide and examine.

5. Note the shape of the red cells; the manner of grouping on the slide; search for nuclei; look for stain in the cells; note the central part of the cell, and measure the cell with the micrometer.

6. In the same preparation examine the white corpuscles which are found adhering to the slide.

Exercise No. 31.—*Use the Haemacytometer, count the cells of both varieties, in a c.m.*

1. Take 995 c.m. of sodium sulphate solution; specific gravity, 1028; temperature, 15 C.

2. Take 5 c.m. of the blood.

3. Mix in the mixing jar with the spud.

4. Take the instrument and fill the cell with the mixed blood and solution.

5. Place the instrument under the microscope and let the whole stand a few minutes; then count the number of cells in ten squares; strike the average; then multiply the average by 10,000, since the blood has

been diluted and since the squares are only one-tenth of a m.m. square. The average number of red cells is about 5,000,000 in each c.m. of blood in men, and 4,500,000 in women.

Exercise No. 32.—Use the Haemoglobinometer.

1. Take the instrument and set it up in front of a piece of white paper.

2. Obtain 20 c.m. of blood and place it in the tube provided for the reception of the blood.

3. Dilute the blood with water until the blood mixture and the standard mixture in the tube coincide in color.

If the blood has the required amount of *haemoglobin* in it, when you have added the water up to the graduate 100, the two colors will coincide. If it reaches the color before the required amount of water has been added, there is not enough haemoglobin in the specimen; and if it requires more water than the 100, there is too much haemoglobin.

Exercise No. 33.—Use the Haematokrit.

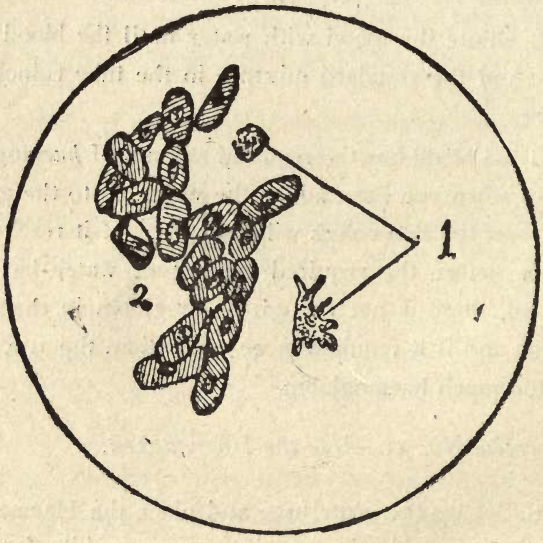
1. Set up the centrifuge and place the Haematokrit in place; revolve the centrifuge very rapidly for three minutes.

2. Observe the position of the heavier materials in the blood.

3. Hold the instrument between you and the light; see how far the red cells register on the graduate. This is a tube of small bore and is arbitrarily graduated. It registers per cent.

Exercise No. 34.—Make a spread of frog's blood.

1. Snip the nose of the animal with a sharp instrument and obtain a small drop of blood.



FROG'S BLOOD.
1, Leukocytes; 2, oval red cells.

2. Collect it in the same way that you did the blood of man.

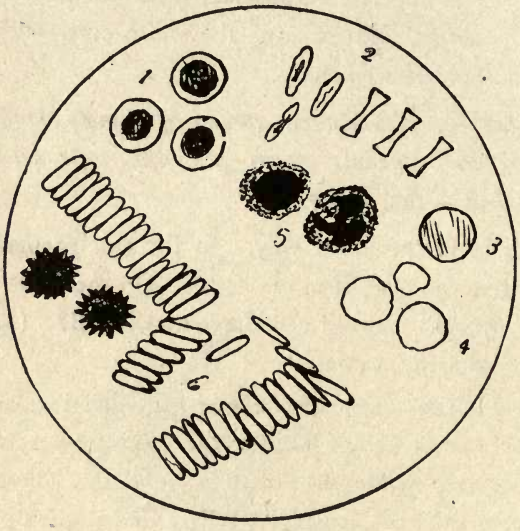
3. Examine the shape of the corpuscles; search for a nucleus in the red corpuscles; measure the cells in both diameters, and examine the white corpuscles for the amoeboid movement.

Exercise No. 35.—Make a spread of human blood and apply the reagents given you, viz.: salt solution, acetic acid, and water.

Irrigate in the usual way. In the salt preparation note what you see; give the cause. Do the same with all the others. Do red cells have a cell-wall? (Leroy and Sobotta say they do.

The colorless corpuscles are of five varieties: *mononuclear lymphocytes, polynuclear lymphocytes, large lymphocytes, eosinophiles* and *basophiles.* These are all larger than the red blood cells. They all originate from the lymph glands. They are separated into their classes by the use of different stains, and the use of the eye-piece micrometer. A large per cent of them has the power of moving like the ameba; hence the term ameboid movement, as applied to the white blood cells. Blood shadows are red blood cells that have been subjected to the action of water, and have the haemoglobin dissolved out, leaving the shadow of the cell. Blood platelets are small red cells. The crystals found in

blood are haemoglobin, haematin, haematoidin. The most important are the hemin, which can be demonstrated by the following method:



HUMAN BLOOD.

1, Biconcave red blood cells; 2, red blood cells on edge; 3, spherical red blood cells; 4, red blood cells after the action of water; 5, leukocytes; 6, rouleaux. Crenated red cells to left of the rouleaux.

Obtain a drop of blood in the usual way, and apply to it some salt solution and some acetic acid; let the whole dry for a few minutes. Examine with H. P.

The red blood cells are derived from the mesoblast, and are nucleated and colorless when first formed.

These multiply and lose their nuclei and acquire hemoglobin. Later in life the spleen and red marrow of the bone contribute to the supply of red cells. The white blood cells are derived mostly from the lymphoid tissue and spleen. The lymphoid corpuscles become dislodged and carried along the blood current or lymph current, as the case may be. "In these places they undergo a mature change."—*Leroy.*

Exercise No. 36.—Make a stained preparation of blood.

1. Make a spread in the usual way.
2. Fix high over the flame.
3. Apply eosin 30 minutes.
4. Haematoxylin 3 minutes.
5. Dry, mount and label.
6. Study and preserve.

Search for the four varieties of lymphocytes and observe the formation of rouleaux in the red cells.

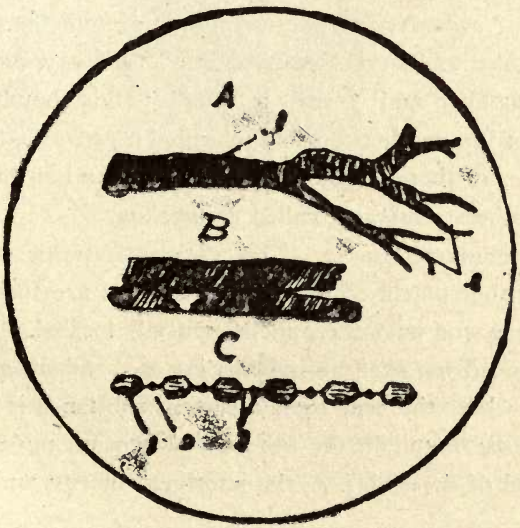
CHAPTER VIII.

MUSCLE TISSUE.

This variety of tissue is of mesoblastic origin, and is divided into three classes: voluntary, involuntary and heart muscle. All muscle tissue is rich in blood vessels, lymphatics and nerves. The blood vessels break up into capillaries, which form a net work around each individual fiber. The nerves terminate in special end-organs under the sarcolemma.

The voluntary or skeletal muscle is the most widely distributed. It forms all the skeletal muscles, and is under the control of the will. It is composed of long, cylindrical fibers, by some authors called cells. These are the largest cells in the body—so large that one cannot be placed in the field of the microscope at once. Sometimes they are 1½ inches long. They are all surrounded by a delicate membrane, or cell-wall, called sarcolemma, just under which are placed the nuclei of the cell. The transverse striations that are seen all along the course, are said to be due to the presence and make-up of the sarcous elements. There are seen running along the long diameter of the cell deli-

cate lines, which are called sarcostyles and can be demonstrated by taking a piece of the tissue and dissociating the same. This will separate the lines into individual fibrils. Each fibril is called a sarcostyle. The space between the sarcous elements is filled with substance, which is called sarcoplasm, and is analogous to the protoplasm in other cells. This protoplasm is endowed with the special power of motion, or contractility. If we use a very H. P. we will be able



SECTION OF VOLUNTARY MUSCLE.

A. Fiber or Cell. 1. Sarcolemma; 2, fibrillae.

B. Two fibers, one showing torn sarcolemma as a clear space.

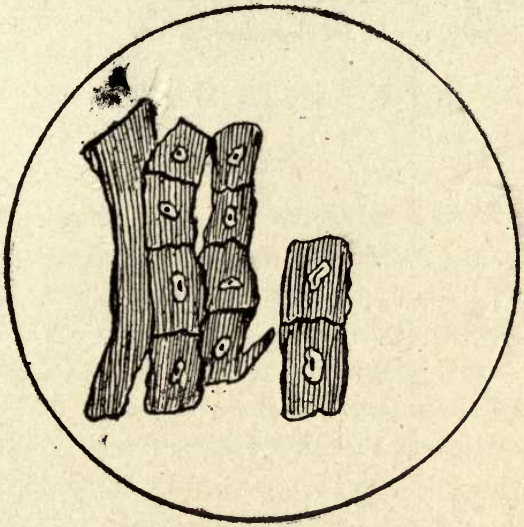
C. A sarcostyle; 1, Fusiform body; 2, a ball; 3, light spaces.

to observe the lines of Hensen and the membrane of Krause. These lines are located in the sarcous elements. The sarcous elements are composed of the following parts, viz.: a spindle-shaped body, light space, and a ball. Placed end to end they make a fiber or cell. The lines of Hensen are those lines seen dividing the spindle-shaped bodies, and the membrane of Krause is the membrane or line drawn across the light bands. The following wrappings are found enveloping the muscle: *endomysium,* the membrane around the fiber. A number of fibers (wrapped in the *endomysium*) is put together and forms a bundle; this bundle is wrapped in another membrane, called a *perimysium.* A number of these bundles is put together and enveloped in another membrane, called *epimysium.*

Involuntary Muscle.—This variety of tissue is not under the control of the will. The cells are fusiform in shape, and have their nuclei centrally located. They are joined together by overlapping each other; at the point where the cells meet a cement substance is seen. They are found in the tissues that are not under the control of the will, *i. e.,* the intestines, uterus, and rectum.

Heart Muscle.—This variety of muscle is found only in the heart, and is not the same as the involuntary muscle, in shape or function. The cells are short, rec-

tangular shape, with branching interlacings, as well as a cement substance between the cells. With a H. P. can be seen a large number of fine transverse striations



L. S. HEART MUSCLE.

in each cell; also a number of longitudinal lines are seen in them. The cell has a single nucleus centrally located. Between each one is found the capillaries and fibrous tissue, which serves to hold the tissue in place.

Exercise No. 37.—Mount some fresh muscle tissue.

Take some teased muscle, place it on the slide and examine. Search for the following parts, and make

drawing: *cells, nuclei,* and the *enveloping membranes.* Proceed in like manner with all three varieties.

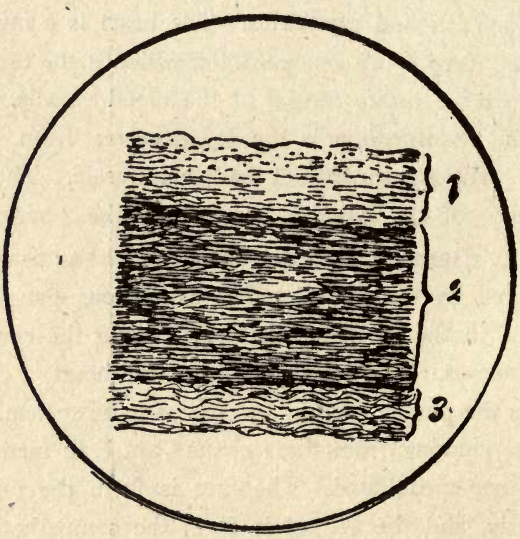
Exercise No. 38.—*Mount the prepared tissue supplied, and study, cover, label and preserve.*

CHAPTER IX.

THE VASCULAR SYSTEM.

The vascular system is composed of the heart, arteries, veins and capillaries. The heart is a muscular *organ, lined by an endocardium,* which is the continuation of the *tunica intima* of the blood vessels. Next to the *endocardium* is the *myocardium* from within out. The *myocardium* is the heart muscle. The outer surface of the heart is covered by the *pericardium:* this is a serous membrane, of white fibrous tissue, and covered by endothelial cells. Numerous ganglia are found in the tissues of the heart, along the course of the nerves. The nerve supply of the heart is derived from the pneumogastric and sympathetic system. The fibers running from these centers are both medullated and non-medullated. The first is from the pneumogastric, and the second is from the sympathetic system. The valves of the heart are simply broad folds of the endocardium, between which are a small amount

of fibrous tissue. The lymphatics of the heart are many and are found in two places, viz.: around the coronary arteries and between the muscle fibers The capillaries are minute ramified arteries, which have lost their outer coat and hence become very thin. This thin space is where the white blood cells leave the vessels in inflammation; the spaces are called stigmata. The blood vessels are richly supplied with lymphatics which are seen ramifying in the outer coat of the larger arteries and surrounding the smaller capillaries.



T. S. BLOOD VESSEL.

Showing bands of yellow elastic tissue.

1, Tunica intima; 2, Tunica media; 3, Tunica adventitia.

The veins are different from the arteries in that they have thinner coats and less muscle tissue; their walls are thinner than the arteries and their lumen is larger. They have more fibrous tissue than the arteries. Arteries are composed of three coats. The *tunica intima*, the *tunica media*, and the *tunica adventitia*. The internal coat is lined with endothelial cells resting upon a basement membrane of white fibrous tissue. Just under this coat is located a band of yellow elastic tissue, called the fenestrated membrane of Henle. The middle coat is composed of the smooth muscle running around the artery, and having a few fibers of fibrous tissue interspersed with the muscle cells, especially the large arteries. The outer coat is composed of white and yellow elastic tissue in abundance. The nerve supply of the artery is derived from the vasomotor system, and the blood supply of a part is regulated by the contraction or expansion of the muscle fibers in the middle coat of the artery. The outer coat is for strength; the inner one for smoothness; the middle one for contraction and expansion. All the blood vessels are of mesoblastic origin. The vessels are closed tubes.

Exercise No. 39.—Make a section of a blood vessel; mount, stain and preserve.

Technic: Use the alcohol hardening method. Observe the coats, and make out the yellow elastic tissue.

Exercise No. 40.—Make a stained preparation of an artery and vein.

CHAPTER X.

THE LYMPH VASCULAR SYSTEM.

The lymphatics are in all particulars like the blood vessels in structure, except that even the largest lympatics have only a small amount of muscle tissue. In all the tissues and organs there are small spaces that are called pericellular spaces. These spaces are the beginning of lymphatic vessels. The small vessels continue to join until they reach the thoraic duct, which pours its contents into the sub-clavian vein: at this point the inner court of the duct is folded over on itself and forms a valve to prevent blood from flowing into the duct. All along the course of the vessels are found similar valves, which prevent the lymph from flowing but one way. While all the tissues of the body are rich in lymph vessels, the ones supplied most are the white fibrous, areola, blood vessels and nerves. In the last two locations they are called perivascular and perineurial lymphatics, respectively. The lymph is a clear fluid, having floating in it a large number of cells and fat granules. The cells are very much like

the small lymphocytes of the blood. The fat is always present in varying amounts, but is increased after a meal. The cells of the lymph eventually become the white blood cells.—*Osburn.*

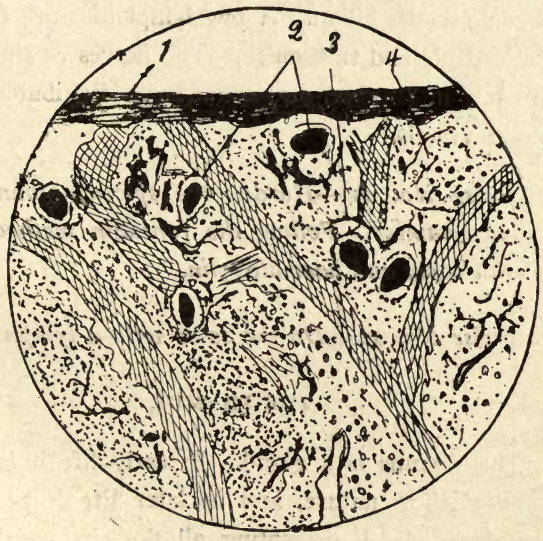
DIFFUSE ADENOID TISSUE.

In many positions throughout the body there are found small nodes of adenoid tissue located in the *tunica propria* of mucous membranes, as in the lungs and digestive tract. This variety of adenoid tissue is called diffused. In some places this tissue is present in small amounts, and fades away into the surrounding tissue without presenting any line of demarcation. In other locations this tissue is surrounded by a band of white fibrous tissue, having dispersed it in a few smooth muscle fibers. This band is called a capsule. The node and band is called a lymph-follicle. When these nodes occur in the course of a lymph vessel, they are called lymph glands. These glands are usually bean-shaped and the capsule sends down little bands of white fibrous tissue, separating the gland into divisions. These bands are called trabecula, and the divisions, lobules. The trabeculae subdivide, forming the retiform tissue of the gland, in which are found the Malpighian corpuscles of the lymph glands. The blood vessels enter the capsules. The nerves of these glands

are of both varieties, viz.: medullated and non-medullated. That portion of the gland which is located near the capsule is called the cortical portion, and that portion near the center of the gland is called the medullary portion.

THE SPLEEN.

The spleen is a lymphoid gland, and differs from the other lymphoid glands in that it has no medullary portion and the blood supply is differently arranged.



SECTION OF SPLEEN.

1, Capsule; 2, Malpighian corpuscles; 3, intralobular vein; 4, splenic pulp. The large bands of fibrous tissue represent the trabeculæ.

The splenic artery enters the spleen at the hilum and passes up the trabeculae for a certain distance, and then leaves them and passes into the splenic pulp; here the artery loses its outer coat and continues as the lymphoid tissue of the spleen. In this place the adenoid tissue collects into a dense mass and the mass is called the Malpighian corpuscle of the spleen. Here the germ center is found. The veins begin where the artery ends and pass to the trabeculae, where they pass out of the organ by following the trabeculae to the hilum. A few lymphatics are found in the capsule and trabeculae. The nerves of the glands are few in number, and are usually distributed to the blood vessels.

Exercise No. 41.—Harden the spleen; mount, stain and examine. Follow the formalin method; stain with eosin and haematoxylin.

Exercise No. 42.—Do the same with a lymph nodule.

THE THYMUS.

The thymus is a gland. In early life it is mostly ephithelial in nature, and in later life it becomes a lymphoid gland, presenting all the characteristics of the spleen. The blood vessels and nerves are found in the trabeculae and send other branches into the

lymphoid tissue. The thymus is the only lymphoid gland that has no germ center, but cell division can be demonstrated in the gland. Hassal's corpuscles are the dense bodies found in the thymus. They are regarded as epithelial remains of the gland in early life.

THE TONSILS.

The tonsils are situated in the mouth at the base of the tongue. They are surrounded by a dense membrane of white fibrous tissue on the under side, and by the mucous membrane, which is made up of pavement epithelial cells of the buccal cavity, on the outer side. The membrane dips down sometimes into the substance of the tonsil, and is called the crypts. The tonsils are supplied with blood vessels, lymphatics and nerves, which enter the organ through the capsule. They are lymphoid bodies.

SEROUS CAVITIES AND MEMBRANES.

The serous membranes may be considered as expanded lymphatics. These membranes are composed of a thin sheet of fibrous tissue, supporting a layer of thin, irregular, polyhedral cells, placed edge to edge and united by a cement substance. At very short distances there are little openings in the mucous membrane, called stomata, which are the mouths of lym-

phatic glands. Sometimes the cells do not adjust themselves completely, and a space is left, called pseudo-stomata. The pleura, pericardium and peritoneum are serous membranes. The serous membranes are not in contact with the air.

THE MUCOUS MEMBRANES.

This variety of tissue is in such a position that it may come in contact with the air. It is composed of the following structures: epithelial cells, basement

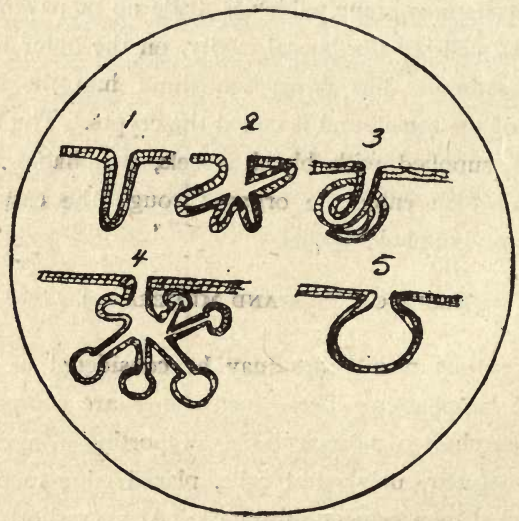


DIAGRAM OF THE DIFFERENT TYPES OF GLANDS.
1, Simple tube; 2, compound tube; 3, simple coiled tube; 4, compound saccular; 5, simple saccular.

membrane, and beneath the basement membrane there is a layer of smooth muscle, called muscularis mucosæ. Along the course of the membrane there are several openings which represent the mouths of glands. These glands are simply an infolding of the mucous membrane. Several types of glands are recognized. All of them are some type of a tube. See cut for types of glands. The different glands will be discussed as they are studied.

CHAPTER XI.

THE NERVES.

The nerves are highly specialized fibers, beginning in a cell and ending in a special end-organ. Their function is to carry impulses to and from the cell. The combination of a cell, end-organ, and nerve fiber, is called a neuron. A nerve cell is one of the largest cells in the body and possesses all the essentials of any cell, viz.: nucleus, nucleolus and protoplasm. They have no cell wall. The cells vary greatly in shape, and each point of the cell is called a pole. One of the points is called an axis cylinder. The points arising from the cell at other points than the one called axis-cylinder are called dendrites. The nerves are named according to the number of processes they have. The first type and second type cells are those cells that have either a long or short axis cylinder. The first is a first type cell, and the second is a second type cell. Neuroglia cells are small cells that give off several short processes, which act as a connective tissue. The cell is called a glia cell, and the fibrils are called neuroglia.

GANGLIA.

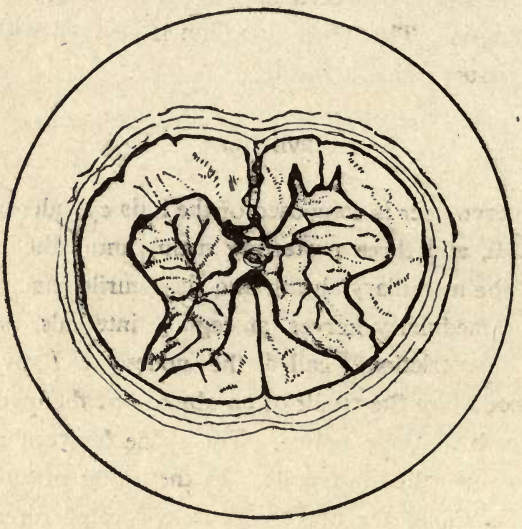
These bodies are nerve centers, consisting of nerve cells and nerve fibers. Some of the fibers end in the ganglion, while others pass on to more distant points. The brain and spinal cord may be termed a group of large ganglia. The whole ganglion is covered with a fibrous tissue, called a sheath.

NERVE FIBER.

The nerve fiber is composed of the axis cylinder of a nerve cell, and three protecting membranes: the axilemma, the medullary sheath and the neurilemma.

In the medullary nerves, at regular intervals, there appear constrictions, called the nodes of Ranvier. These nodes are the result of an absence of the medullary sheath at these points. The space between any two nodes is called internode. In the center of an internode is found the nerve corpuscle, located directly under the neurilemma. The non-medullated nerve fibers are found in the sympathetic system. Each fiber consists of an axis cylinder, neurilemma and an oval nucleus upon the surface of the fiber. There are two theories concerning the formation of the axis cylinder: one is that the nerve cells fuse themselves together, end to end, and form a long thread; the other is that

the nerve cells have a large number of processes, and one of these functions as an axis cylinder. In both cases the neurilemma corresponds to a cell membrane. A medullated fiber is a fiber with a medullary sheath, and a non-medullated fiber is a fiber without a medul-



T. S. SPINAL CORD.

Showing investing membranes and distribution of blood vessels in the white and gray substances.

lary sheath. The nerves end in the muscle tissue by expanding into a small plate of nerve fiber. There are two main divisions of nerves, viz.: central and sympathetic nerves. We will study each of these in detail.

The central nerves are studied by taking a section of the spinal cord and dissociating the tissue, then scraping the anterior cornua and spreading the material on a slide and examining it in the fresh state. The best method is to study a stained section and compare results with the cuts in all good books on the subject.

Exercise No. 43.—*Take a T. S. of the spinal cord of a dog and scrape the anterior cornua, and examine the scrapings.*

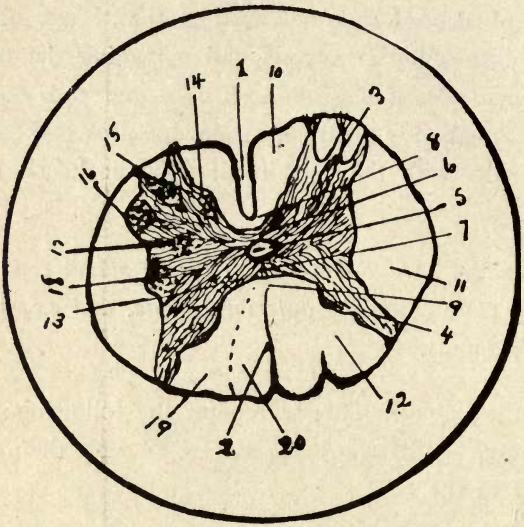
Use the saline mount; search for the following: A large nerve cell having its processes. Locate the axis-cylinder of the cell.

Exercise No. 44.—*Examine the nerve of a dog or ox in salt solution.*

Technic same as above.

Exercise No. 45.—*Make a T. S. of spinal cord from tissue that has been hardened. Stain, mount and preserve.*

Search for the following: the gray matter, consisting of ganglion cells, neuroglia and fibers; the white matter, consisting of medullated fibers and connective tissue. The white matter surrounds the gray. The gray



T. S. SPINAL CORD—CAT.

1, Anterior median fissure; 2, posterior median fissure; 3, anterior roots; 4, posterior roots; 5, central canal; 6, anterior gray commissure; 7, posterior gray commissure; 8, anterior white commissure; 9, posterior white commissure; 10, anterior columns; 11, posterior lateral column; 12, posterior column; 13, substantia gelatinosa; 14, 15, 16, 17, 18, groups of motor cells; 19, column of Burdoch; 20, column of Goll.

matter is in the shape of the letter H. The surface of the cord is divided into four areas: anterior, posterior and two lateral. On dividing the cord into halves, from before backwards, and then dividing

each half into three columns, we have the anterior, posterior and lateral columns of each half, making six columns in the cord. In the lower cervical and upper thoracic region two more divisions appear in the posterior column, the column of Goll (placed near the posterior fissure) and the column of Burdoch. The white matter is connective tissue and nerve fibers, having a large number of glia cells interspersed in them. The nerve fibers have a medullary sheath, but no neurilemma. "The nerve fibers run in the same direction as the long axis of the spinal cord; a few may be seen which run across the cord."—*Leroy.* Around the entire cord are found three protecting membranes: the dura mater, arachnoid and pia mater. The space between the dura mater and arachnoid is called the subdural space. The space between the arachnoid and pia mater is called the sub-arachnoidal space. All these membranes are composed of white fibrous tissue and endothelial cells. They are all more or less connected by fine fibers of fibrous tissue, which run transversely to the long axis of the cord. The Pacchionian bodies are large papillae projecting upward into the subdural space. The blood vessels are found in the dura mater and pia mater; but none are found in the arachnoid. They are most numerous in the pia mater.

The gray matter of the cord is arranged in the shape of the letter H. The long arms of the H are directed

from before backwards, and the short arm is directed from side to side, joining the long arms at their middle points. The long arms are comma-shaped, with the big end of the comma directed anteriorly, *i. e.*, towards the front of the cord. This end does not approach the surface as near as the small end of the comma. The small end is called the posterior horn, while the large end is called the anterior horn of the cord. In the center of the transverse arm of the H. is seen a small opening, called central canal, lined with ciliated epithelial cells, and containing a fluid called cerebro spinal fluid. The transverse arm of the H is called the *gray commissure,* and the white matter immediately in front and behind it is called respectively *anterior* and *posterior white commissure.* Some histologists divide the gray commissure into anterior and posterior, with reference to *central canal.* There is a narrow cleft in the white substance, beginning at the anterior median periphery, and extending to within a short distance of the gray commissure, called *anterior median fissure.* A narrower but similar fissure is seen on the posterior side of the gray commissure, called *posterior median fissure.* On each limb of the letter H of the cord there are three horns; anterior, posterior and lateral horns, located as their names indicate. From the anterior horn emerge the anterior roots, and the posterior horns receive the pos-

terior roots. From the lateral horn emerge the motor nerve axis-cylinders, the same as in the anterior horn. The nerve cells of the anterior horn resemble a straightened comma, with a very long tail. The reticular processes, column of Clark, and the substantia gelatinosa, enter the structure of the posterior horn. The *substantia gelatinosa* covers the horn and surrounds the central canal. The nerves of the anterior horn and the column of Clark are very large and stellate. The blood vessels of the cord enter it mostly from the pia mater, and branch repeatedly to form capillaries, which run parallel with the fibers in the white matter, but form a net work in the gray matter. In both cases, they are surrounded by neuroglia tissue. There are thirty-one pairs of nerves springing from the spinal cord. The motor nerves are anterior, while the sensory nerves are posterior. They are distributed to the skin and other parts of the body, and terminate in a special nerve-ending.

Exercise No. 46.—Make a T. S. of the spinal cord.

Technic: Muller's fluid, paraffine, hæmatoxylon and eosin.

Locate the following: (L. P.) Enclosing membranes, median fissures, gray matter, white matter, axis-cylinders in the white matter, T. S. with their en-

closing membranes, the horns of the cord in the gray matter, the different shaped and sized nerve cells in the anterior horn, central canal, anterior and posterior roots, the different commissures, and the entrance of the blood vessels in the pia mater.

CEREBRUM.

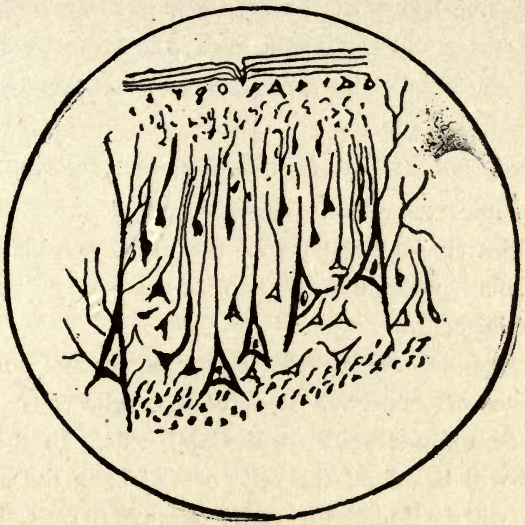
The cerebrum is composed of five layers of nerve cells, which merge imperceptibly into each other. They are:

1. The molecular layer. This is the first layer, and is composed of a number of finely granular cells, with medullated fibers and neuroglia. The cells of Cajal are found in this layer.

2. The layer of small pyramidal cells. This is located under the first layer and the processes that leave the cells form a net work. The axis-cylinders leave the basal ends of the cells and send collaterals in the medulla. The dendrites enter the molecular layer.

3. The layer of large pyramidal cells. This differs from the second only in size of the cells, and in this layer there is no network sent to the layer above.

4. The layer of irregular cells. This layer is composed of oval or polygonal cells, which have no special dendrites. Each axis-cylinder sends out collaterals, and enters the medulla to become one or two nerve fibers.



SECTION OF CEREBRUM.

Showing morphology and arrangement of the cells.

5. Spindle-shaped cells. This layer lies next to the medulla, and is composed of spindle-shaped cells, with nerve fibers between them. The cells are arranged parallel with the fibers. These five layers constitute the cortex of the cerebrum. In these, that part of the nervous force dedicated to the cerebrum, is carried on. The fibers extending from the cortex, with the connective tissue structure holding the nerve fibers together, constitute the medulla of the cerebrum.

The five layers of the cerebrum are the molecular layer, the small pyramidal layer, the large pyramidal layer, the layer of irregular cells and the layer of spindle-shaped cells.

Exercise No. 47.—Make a T. S. of the cerebrum.

Technic: same as the spinal cord.

1. Search for the layer of the granular cells, large and small pyramidal cells, and the layer of irregular and oval cells.

2. Examine the medulla of the cerebrum; note the presence of connective tissue cells, or glia cells.

3. Search for spider and Cajal cells. In the first layer will be found the cells of Cajal; in the second layer will be found the small pyramidal cells; in the third layer will be found the large pyramidal cells; in the fourth layer will be found the irregular cells; in the fifth layer will be found the oval cells.

CEREBELLUM.

The cerebellum is composed of three layers: The outer molecular, the middle granular or rust colored layer, and the inner medullary tract. The outer layer consists of three varieties of nerve cells: the cells of Purkinje, the polypolar or multipolar, and the stellate cells. The middle layer contains two varieties of cells: the granular cells and the short stellate cells. The inner layer contains two varieties of cells: the centrifu-

gal neuraxis of the Purkinje cell and the two types of centripetal cells, one type sending their neuraxis into the granular layer. These fibers are called mossy fibers and do not extend through the granular layer. The other type of cells sends the collaterals of the cells into the layer where the Purkinje cells are found and seem to ascend the neuraxis of the Purkinje cells, and are called climbing fibers of the centripetal cells. The central gray nucleus are found in the medullary portion of the cerebellum, and are a number of multipolar cells with many dendrites. The medullary or white substance of the cerebellum is arranged in a layer of branching sheets, and when cut, presents in the cut section a tree-like arrangement, called arbor vitae. For more extensive work on this subject see Leroy, Sobotta, and Bohm and Davidoff.

THE MEDULLA OBLONGATA.

This structure is the same as that of the spinal cord, the chief difference being in the arrangement of the structures.

SYSTEM OF SYMPATHETIC NERVES.

This system is composed of a large number of ganglia and nerve fibers, connected by small fibers. The fibers are non-medullated. They are arranged into

three large groups, viz.: the cardiac ganglia, the epigastric ganglia and the hypogastric ganglia. These ganglia are located in front of the spinal column.

NERVE ENDINGS.

There are three varieties of nerve endings: free nerve endings occurring in the skin, mouth, spinal cord, etc., terminal corpuscles occurring in the skin, and neuro-epithelial, occurring in the perceptive organs, viz.: mouth, eye, nose and ear.

Exercise No. 48.—Examine the skin T. S.

Technic: Harden in alcohol, embed in celloidin, and stain with hæmatoxylin and eosin.

1. Search for the four layers of the cutis-notha, hair follicle, hair in place, hair muscle, sweat gland, sebaceous gland, nerve endings and pigment of the skin.

In what layers are all these objects found?

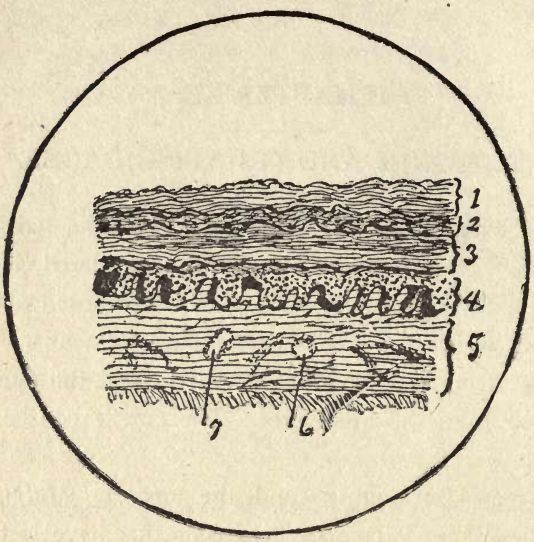
A sweat gland is a simple tubular gland.

What lesson do you get from the skin bearing on the nervous system?

CHAPTER XII.

THE SKIN AND ITS APPENDAGES.

The cutaneous system comprises the skin, hair and glands. The teeth are sometimes considered as belonging to this system. The skin is composed of two distinct structures, viz.: the cuticle and cutis. The cuticle is derived from the ectoderm and the cutis is derived from the mesoderm. The cuticle consists of four layers; the *stratum corneum,* the *stratum lucidum,* the *stratum granulosum* and the *stratum Malpighii.* The first layer is composed of cells that have lost the appearance of ever having been nucleated, called the cornified layer. The second layer is composed of cells, which are polyhedral in shape and have almost lost their nuclei. The third layer is composed of cells irregularly polyhedral in shape and are joined together by little spines. These cells are called prickle cells, and outside of these are seen other cells, which have a granular protoplasm. These two layers constitute the stratum granulosum. The fourth layer is composed of cells, which are columnar in shape and are in a stage of active growth, as shown by the property they



L. S. SKIN.—HUMAN.

1, stratum corneum; 2, stratum lucidum; 3, stratum granulosum; 4, stratum Malpighii; 5, corium; 6 and 7, vessels of the skin.

have of straining so easily. This layer is the one that contains the pigment that gives color to the skin in the colored races and, in some locations, in the white race. This layer rests upon the true skin. The true skin is composed of two layers of fibro-elastic tissue, called papillae of the true skin. The nerves end in the true skin, in end corpuscles, and in the cuticle in an arboriztion manner.

THE SEBACEOUS GLANDS.

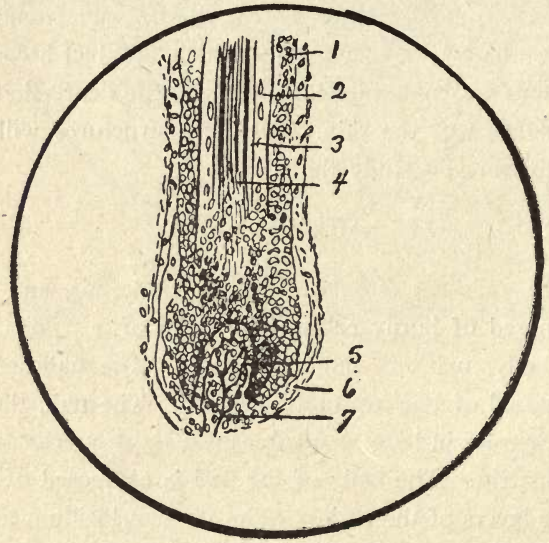
These glands are of the compound saccular type and are located in the papillæ of the corium. They consist of acini, secreting cells and a duct. The sudoriferous glands belong to the tubular type, and consist of a basement membrane and gland cells and a duct. The gland is located deep down in the tissue beneath the skin. The deep end is coiled upon itself and the superficial end is straight nearly all the way from the coil to the cuticle. Just at the cuticle the duct becomes tortuous and makes its way out as a little depression on the surface of the skin. All these structures will be seen when you study the skin.

NAILS AND HAIR.

The nails are derived from the epidermis, and are composed of horny cells. The parts of a nail are nail-body, nail-bed and nail-matrix. The nail-bed is composed of the stratum Malpighii. The nail, therefore, grows in length and thickness by new cells from the matrix. The body of the nail is composed of the other layers of the cuticle. The stratum lucidum takes the greater part in the formation of the nail as the transparency indicates.

The hair, like the nail, is derived from the skin, and is found almost all over the body. There are no hairs

in the palms of the hands and soles of the feet. The hair consists of three divisions: the shaft, that part above the skin; the root, that part in the skin; the bulb, that part from which the hair grows—in fact, it is a part of the root. The hole in which the hair rests is called the follicle. The shaft consists of three layers: the corticle, cortical and medulla. The cuticle is the outermost covering and consists of epithelial



L. S. HUMAN HAIR.

1, cuticle; 2, inner root sheath; 3, Henle's layer; 4, medulla of hair; 5, papillae of hair; 6, hair bulb; 7, vessels of hair.

cells overlapping each other. The cortical is the thickest and contains the coloring matter of the hair. The medulla is not always present, but when it is, it contains air and gives an appearance according to the light used to examine the specimen.

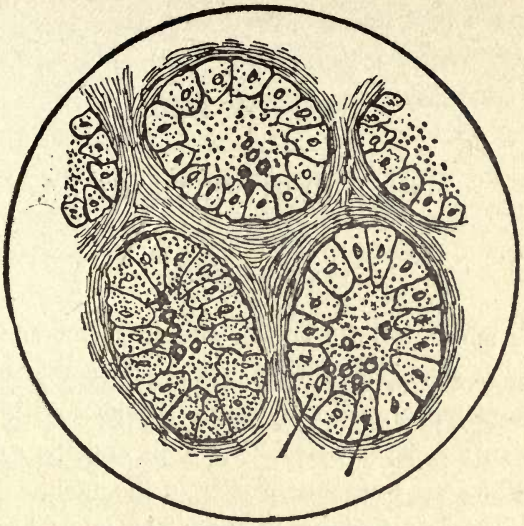
At the bottom of the hair follicle is the fibrous tissue that contains the blood-vessels of the hair. The sheaths of the hair are named according to their location. The arrectores muscles of the hair are composed of a few fibers of smooth muscle-tissue arising in the fibrous of the corium, and cause the hair to stand on ends in case of fright. A longitudinal section of the hair presents for examination: shaft, composed of three layers of modified epithelial cells; root, that part of the hair within the skin; the bulb, a division of the root; the papillæ, an elevation of the true skin into the root of the hair; the sheaths, surrounding the root and bulb, called root sheaths (the opening and the sheaths are called the hair follicle); sebaceous glands, composed of a saccular variety of glands, located about midway the root of the hair.

The theories as to the growth of hair are two: first, that the hair grows from an infolding of the epidermis of the skin; second, that the hair grows from the papillæ at the bottom of the root. The nerves of the hair are few and extend only to the duct of the sebaceous

gland. There is only one fiber to each hair. The shedding of the hair is a difficult subject to understand, but the theory is that the cells in the papillæ harden and therefore the hair loosens and falls out. These are called bulb hairs. The papillary hairs are shed in this way from the external root sheath there arises a bud, which grows downward and develops into a new hair, which gradually pushes the old hair out. The condition called goose flesh is caused by a contraction of the muscles of the hair. The nerves supplying the muscles of the hair are derived from the sympathetic nerves and are called pilomotor nerves.

THE MAMMARY GLANDS.

These glands are included in the cutaneous glands. They are a variety of compound saccular glands, and are histologically considered as a tube being lined with a basement membrane and glandular epithelial cells with large nuclei. The opening in the gland is called acinus. The end of the tube is called the duct. A large number of these glands is put together and forms a lobule having a small amount of white fibrous and connective tissue placed around it. A large number of these lobules is put together and forms a lobe. The gland, as we see it, is a number of glands—*i. e.*, just as the liver is composed of separate livers, so is



T. S. MAMMARY GLAND.
Showing lobules, connective tissue, and acini lined with columnar epithelial cells.

this gland. The mammary is richly supplied with blood vessels and lymphatics. The lymphatices of this gland communicate with those of the axilla. The secretion of it is called milk. Milk is a mixture of fat, leukocytes and colostrum corpuscles.

Exercise No. 49.—Make a T. S. of the mammary gland.

Technic: Formalin, celloidin and H. & E.

Look for the lobe, lobule, acinus and connective tissue elements.

CHAPTER XIII.

THE DIGESTIVE TRACT.

THE MOUTH.

The mouth is lined with a mucous membrane consisting of stratified epithelial cells. Upon the surface of the mouth are seen the ducts of numerous glands. Between this layer of epithelial cells and the submucous tissue are found the papillæ of the mouth. They are composed of fibrous tissue. The gums have no glands in them. The blood supply is derived from the mucous membrane blood supply. The lymphatics are from the same source.

THE TEETH.

The teeth are said by some to be a part of the tegumentary system. The structure of a grown tooth is as follows: every tooth has a *neck, crown* and *root.* The crown is composed of a modified epithelial cell tissue, called enamel. The enamel is the hardest substance in the body, and is covered by a membrane of epithelial

cells, the *cuticula dentis.* The enamel substance is arranged in columns or prisms called enamel prisms. They are stuck together with a substance more dense than themselves, called enamel-cement. The lines of Retzius are parallel lines running from the dentine to the *cuticula dentis* in the enamel. They represent the periodic deposition of the salts of lime in the enamel.

THE DENTINE.

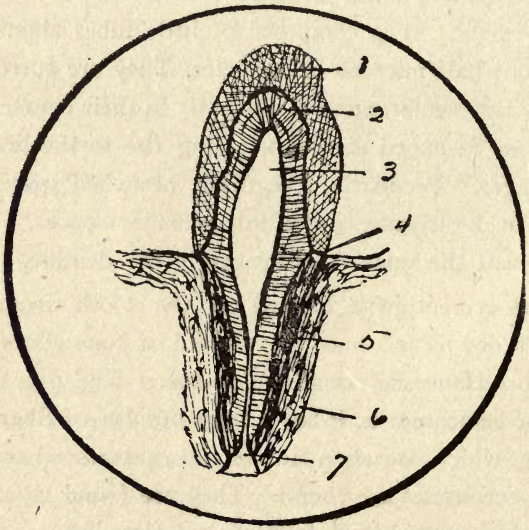
The dentine is the tissue between the enamel and the pulp-cavity. It is composed of little tubes about two and one-half microns in diameter. They are surrounded by the membrane of Newmann. In their course they take an S-shaped direction, giving rise to the lines of Schrager. Peculiarly, irregularly branched spaces are seen in the dentine, called interglobular spaces. They represent the uncalcified spaces in the dentine.

The cementum is located on the tooth from the crown downward, and is composed of bone plates having no Haversian canals as a rule. The peculiarity of the cementum is, it has a large number of Sharpey's fibers, which are abundant in those arears where noe bone corpuscles are found. They are found where the bone has not calcified.—*Bohm and Davidoff.*

The pulp cavity is the cavity found in the central portion of the tooth, and contains the pulp. It is very

large. The pulp is composed of fibrous tissue, nerve fibers, a semifluid substance and an artery. The artery enters the cavity and breaks up into capillaries. The nerves supply the tooth substance by the branching method.

The epiblast and hypoblast, both enter into the composition of the tooth. The epithelial of the primitive jaw becomes thickened and grows down into the subjacent tissue and forms the dental ridge. From the lower outer border of this flask-shaped mass of cells,



L. S. TOOTH.

1, enamel; 2, dentine; 3, pulp cavity; 4, neck of the tooth; 5, cementum; 6, pericementum; 7, apical foramen.

dental bulbs grow (one for each milk tooth), and subsequently become hollow on the under surface and cover a conoid upgrowth of connective tissue cells (the papillæ). The dental bulbs continue to expand one for each tooth, assuming the form of the crown of the tooth, and the inner layer of the cells become columnar. Each of these columnar cells secretes an enamel prism, and the bulb is now called the enamel organ. "While this has been in progress the outer cells of the papillæ have elongated to form the odontoblasts which deposit the dentin. From what has been said, it will be seen that the enamel is deposited outwards and the dentin inwards. The remains of the papillæ become the pulp of the tooth. The permanent teeth are formed similarly. The cementum is deposited later by the alveolar periosteum."—*Leroy.*

Exercise No. 50—*Examine the L. S. of the tooth supplied.*

Make out the structure enamel.

Exercise No. 51.—*Examine the T. S. of the tooth.*

ESOPHAGUS.

The esophagus is composed of four coats, viz.: mucous, sub mucous, muscularis mucosae, muscular and fibrous coat.

The mucous coat is composed of stratified epithelial cells resting upon a basement membrane.

The muscularis mucosæ is composed of a band of smooth muscle located just under the mucous coat.

The submucous coat consists of fibrous tissue, lymphatics, nerves, blood vessels and glands, which empty their contents into the esophagus.

The muscular coat is composed of two layers of muscle tissue, the inner layer being placed circularly and the outer layer placed longitudinally. The upper third is composed of the involuntary variety.

The fibrous coat is composed of a thin layer of white fibrous tissue and a few fibers of yellow elastic tissue.

Exercise No. 52.—*Make a T. S. of the esophagus.*

Technic: Formalin, celloidin and H. & E.

Make out all the coats and describe the tissue in each.

STOMACH.

The stomach consists of the usual four coats: mucous, submucous, muscular and fibrous. The *muscularis mucosae* belongs to the mucous coat.

The mucous coat consists of a basement membrane resting upon a stroma of connective tissue, and supporting a layer of columnar epithelial cells. In this

layer of cells are found a few goblet cells. The glands of the stomach are of two varieties; the peptic and pyloric. The peptic are of the tubular variety, while the pyloric are of the compound tubular variety, and are located in the pyloric end of the stomach. Under the microscope the type of glands will tell what end of the stomach is under consideration. The peptic glands secrete pepsin, and the gastric glands secrete the acid of the stomach.

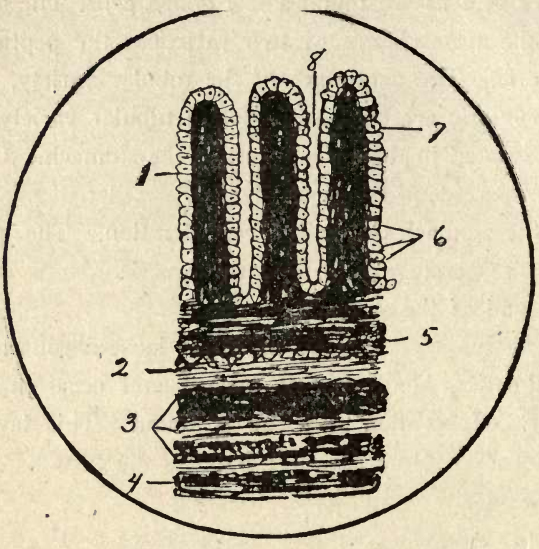
The submucous coat consists of loose fibrous tissue, lymphatics, blood vessels, nerves and occasionally a little diffuse adenoid tissue is found. This layer is thrown up into folds, forming the rugae and depressions of the stomach.

The muscular coat consists of three coats of muscle tissue; an inner circular, a middle longitudinal and an outer oblique layer.

The fibrous coat is made up of white fibrous tissue derived from the peritoneum, and is covered on the outside with endothelial cells. The outer coat of muscle tissue is usually absent. The lymph-follicles are not numerous in the stomach.

THE SMALL INTESTINES.

The small intestines consist of the usual four coats, arranged in the usual manner. The folds in the intestines are called villi, and are composed of the same



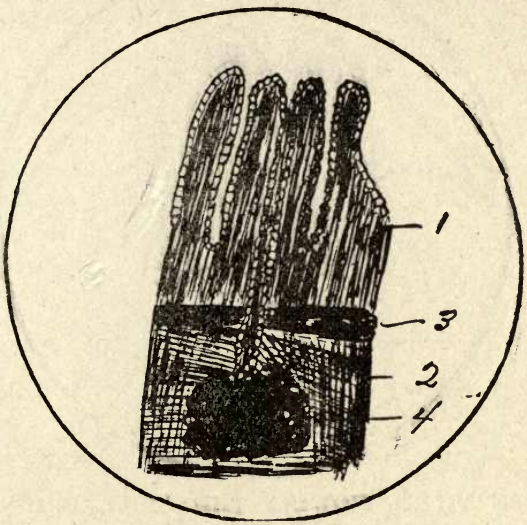
T. S. SMALL INTESTINES.

1, mucosa, covered with columnar epithelial cells; 2, submucosa; 3, muscular layers; 4, fibrous layer.

tissues as the rugæ of the stomach. The goblet cells are abundant in this tissue. In this coat there are a few smooth muscle fibers which give rise to the *muscularis mucosae*. The submucous coat is the same as that of the stomach.

The muscular coat is made up of two layers: an inner circular layer and an outer longitudinal layer, separated by a small amount of connective tissue.

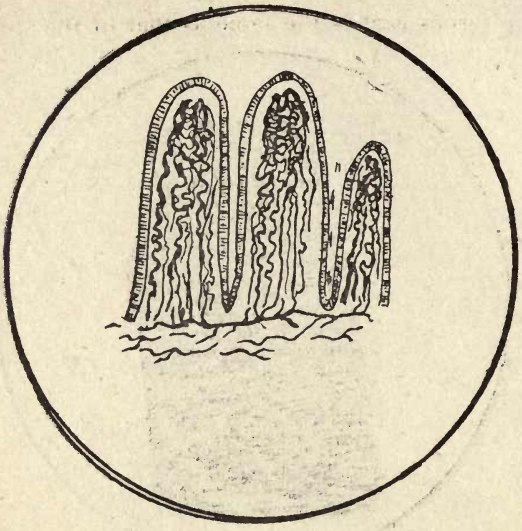
The serous coat is the same as that of the stomach.



SMALL INTESTINE.—CAT.

1, villus; 2, submucosa; 3, muscularis mucosae; 4, Brunner's gland located in the submucosa.

The glands of the small intestines are of four varieties: *Lieberkuhn's glands* are found in the mucosa of the entire intestinal tract, between the villi. They are of the simple tubular type. The glands of *Brunner* are of the same type as the ones above, but divide into many branches; therefore they look like a racemose gland. They are serous and not of mucous type. These are the duodenal glands. The *solitary glands* are found throughout the intestinal tract and are lymph



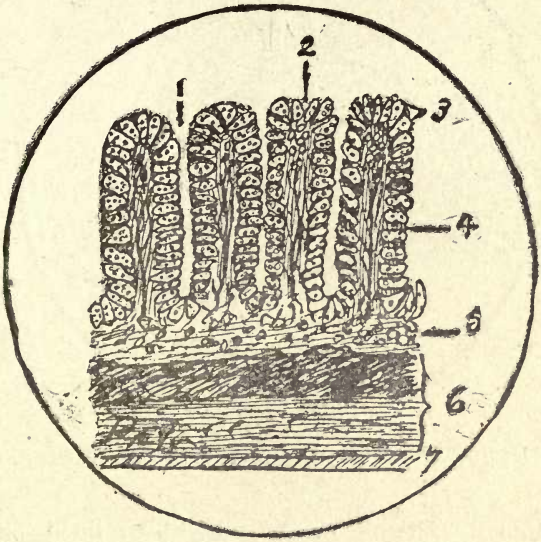
THREE VILLI, SHOWING THE ARRANGEMENT OF THE VESSELS.

follicles. *"Peyer's patches* are elongated structures consisting of from ten to sixty adjacent solitary follicles connected by their cortical zones."—*Sobotta.* They occur in the mucosa and submucosa. The nerve supply of the intestinal tract is derived from the sympathetic and cranial nerves.

Exercise No. 53.—Examine the following: esophagus, stomach, small intestines and large intestines.

Technic: Corrosive sublimate and celloidin. Stain with H. Car. and H. & E.

THE LARGE INTESTINES.

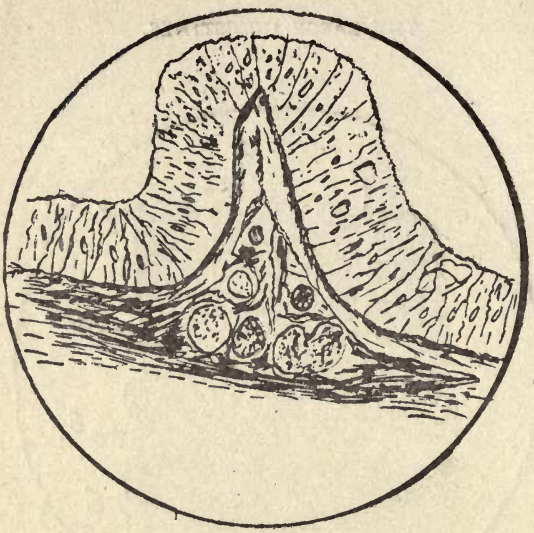


SECTION OF DOG'S LARGE INTESTINE.—PREPARED BY A. L. PAEY.

1, lumen of gland; 2, columnar epithelial cells; 3, goblet cells; 4, mucosa; 5, submucosa; 6; muscular layers; 7, fibrous layer.

THE LARGE INTESTINES.

The large intestines differ only from the small in that they have a fewer number of glands in them. The lymph follicles and the glands of Lieberkuhn are the



T. S. LARGE INTESTINES SHOWING LYMPH NODULES.

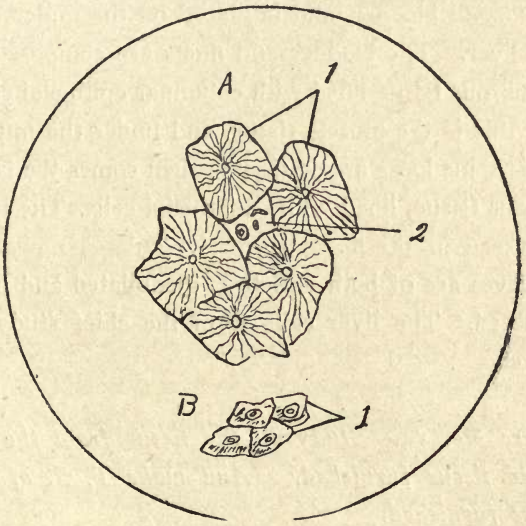
ones found. The rectum is lined with stratified epithelial. Its mucous membrane is thrown into folds called rectal valves.

THE LIVER.

The liver is composed of lobes and lobules, having varying amounts of connective tissue between each lobule and between each lobe. This fibrous tissue is called the capsule of Glisson. A lobule of the liver is composed of the following: beginning, in the center, you find the intralobular vein; surrounding this (with

H. P.), you see the liver cells; following these to the outer edge of the lobule, you see the interlobular vein enclosed in a few fibers of Glisson's capsule. At the same point you see the bile capillaries leaving the lobule.

The blood of the interlobular branch of the hepatic artery and the blood of the interlobular branch of the portal vein mix and pass into the lobule between two lobules, called the interlobular vein, and then pass to the center and out of the lobule through the intralobu-



A.—T. S. LIVER—CAT.

1, lobules; 2, interlobular connective tissue.

B.—1, liver cells.

lar vein. A number of these intralobular veins unite to form the sublobular vein, and these again unite to form the hepatic veins, which carry the blood to the inferior vena cava. Between each liver cell are seen the beginning of the bile capillaries, which pass out to the edge of the lobule and empty into the bile ducts in that place. The bile ducts unite to form the large bile duct. The large duct passes on to the duodenum. When there is more bile than wanted for the digestive tract, the bile is backed into the bile cyst. This cyst is called gall bladder, and is placed on the under side of the liver. The bladder and ducts are composed of white fibrous tissue lined with columnar epithelial cells. Under this is the muscle tissue, and under the muscle tissue is some loose areola tissue—then comes the layer of fibrous tissue, lined with endothelial cells. The lymphatics are in the form of perivascular lymphatics. The nerves are of both varieties: medullated and non-medullated. The liver cells form the chief study of the liver.

Exercise No. 54.—*Study all the tissue from the injected tissue furnished. And make T. S. of the tissue furnished.*

Stain, mount, study and preserve.

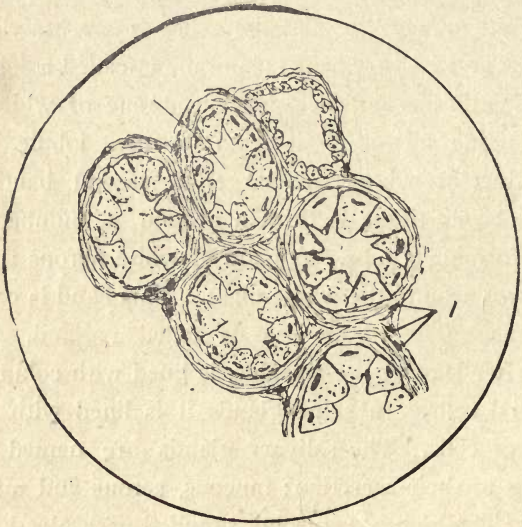
THE SALIVARY GLANDS.

The salivary glands are the parotid, submaxillary, sublingual and the pancreas. They are all of a true glandular nature, having a duct, which divides and subdivides until it reaches the alveolus of the gland. The little alveolus is in reality the lumen of the duct lined with glandular epithelial cells. Each individual alveolus and its basement membrane is called a lobule. Each lobule has an enclosing membrane of white fibrous tissue separating it from the next lobule. As these duct branches grow larger a second band of white fibrous tissue is thrown around a number of them, forming a lobe. A band of white fibrous tissue is thrown around the whole mass. This band is called a capsule. The whole mass is called a gland. The duct, after leaving the alveolus, is lined with columnar epithelial cells. In some glands it is lined with two layers of cells. The salivary glands are named according to their secretion: mucous, serous and mixed glands. The glands are richly supplied with blood vessels. The artery enters the gland at the point where the duct leaves it, and when it reaches the acini it breaks up into a network of capillaries that reunite to form the veins, which leave the gland, accompanied by the artery. The lymphatics are found with the artery as far as the acini. The nerves are of both varieties and

reach as far as the acini. There are found small ganglia in the glands.

THE PAROTID GLAND.

This gland is a serous gland. It presents for an examination a capsule, septa of connective tissue, lobes



T. S. SUBMAXILLARY GLAND.

1, fibrous connective tissue between the lobules.

and lobules. The lobe is composed of lobules, connective tissue between the lobules and a duct. The lobule contains acini and ductules. The acini is the terminal of the tube of which the gland is composed. The com-

position of acini: glandular cells, resting on a basement membrane and the lumen of the tube, or gland. The ductules discharge their contents into the ducts.

Exercise No. 55.—Study, mount and preserve the section supplied.

Note the method of stain used in your book.

Exercise No. 56.—Make a study of the pancreas in the same way you did the parotid.

Note the difference, if there is any. The ducts of all glands are lined with columnar epithelial cells. Sometimes the cells are arranged in two layers. (See the scheme of the salivary-glands in *Bohm and Davidoff,* page 227.)

Exercise No. 57.—Make a study of the gland tissue supplied and make out all the parts named as belonging to a gland.

Mount and preserve.

Exercise No. 58.—Make a T. S. of all the glands of the mouth and note the difference, if any, in them.

Make drawings of all of them. There are numerous small glands in the lips and oral cavity generally, and are named according to their location.

Exercise No. 59.—Make T. S. of the lip.

Study, mount and preserve.

THE PANCREAS.

The pancreas is the abdominal salivary gland and is

SECTION OF PANCREAS.

in structure the same as the other glands of the same name.

Exercise No. 60.—Make a T. S. of the pancreas.

Study, mount and preserve.
Note the technic in all cases.

CHAPTER XIV.

THE URINARY TRACT.

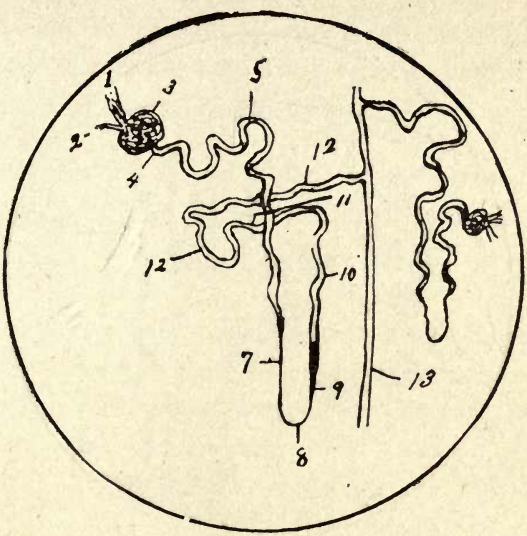
THE KIDNEY.

This organ is a compound tubular gland, consisting of about twenty lobules, surrounded by a dense layer of white fibrous tissue called a capsule. In this capsule is found some smooth muscle tissue. The kidney is of a bean shape. And the hilum is directed towards the median line of the abdominal cavity. At the hilum the artery enters, and the vein and ureters leave. The nerves are of both kinds: the medullated and non-medullated.

The outer investment is called a capsule, having in it a few smooth muscle fibers. The cortex of the kidney is located just under the capsule and constitutes one-third of the kidney substance. It contains the labyrinth, Malpighian bodies and uriniferous tubules. The labyrinth is the space found between the upper end of the uriniferous tubules, and extends downwards to the point where they all unite to empty into

the calyces of the kidney. It will be remembered that the uriniferous tubules are gathered into bundles and then proceed downwards until they reach a point just above the calyces, where they join several other bundles forming a mass of tubules which empty into the calyces. There are several such masses in one kidney. These masses are called the medullary substance of the kidney. The beginning of the medullary substance is nearly like the fingers of your hand. Hold your hand up before you and both extend and separate your fingers. You will then have a fair example of the medullary rays, medullary substance, labyrinth and tube of Bellini, the fingers representing the medullary rays; the spaces between them representing the labyrinth, the palms representing the medullary substance and the wrist the tube of Bellini. The tube of Bellini is the excretory duct of the uriniferous tubules.

The blood vessels enter the kidney at the hilum and pass upwards and give off branches to form the glomerulus. The glomerulus is a coil of an artery, which enters the capsule of Bowman at the same point where the vein leaves the capsule. In this body the urine is separated, in part, from the blood. (For the theory of the secretion of urine, see the text on Urine.) The uriniferous tubule is the tube which carries the urine from the glomerulus to the calyces of the kidney, and it



SCHEME OF URINIFEROUS TUBULES AND GLOMERULI.

1, afferent artery; 2, efferent vein; 3, glomerulus; 4, neck of the uriniferous tubule; 5, proximal convoluted tubule; 6, spiral tubule; 7, descending limb of Henle's loop; 8, Henle's loop; 9, ascending limb of Henle's loop; 10, spiral portion of the ascending limb; 11, zigzag tubule; 12, distal convoluted tubule; 13, straight collecting tubule.

leaves the glomerulus at the opposite pole from the artery and vein. The uriniferous tubule takes a tortuous course and has received names accordingly: 1, the neck, lined with low cuboidal epithelial cells; 2, the convoluted tubule, lined with low columnar cells; 3, the spiral tubule, lined with the same as No. 2; 4, the descending limb of Henle, lined with squamous cells;

DIAGRAM OF KIDNEY.

1, intralobular artery; 2, glomerulus; 3, afferent vessel (vein) 5, intralobular vein; 6, venous capillaries of the cortex; straight uriniferous tubule.

5, Henle's loop, lined with squamous cells; 6, the ascending limb of Henle, lined with the same sort of cells as the loop; 7, the irregular tubule, lined with striated columnar epithelial cells; 8, the distal convoluted tubule, lined with granular epithelial cells; 9 the arched collecting tubule, lined with low cuboidal cells; 10, the straight collecting tubule, lined with columnar cells; 11, the excretory duct, lined with tall columnar cells.

The sinus of the kidney is formed by the union of the excretory duct and is lined with white fibrous tissue of the tubules, and is continuous with the ureters.

Exercise No. 61.—Make sections of the tissue given you and study, mount and preserve.

URETERS.

The ureters are the two tubes that convey the urine from the kidneys to the bladder and are composed of three coats; viz: mucous, muscular and fibrous. They are lined with squamous cells.

THE BLADDER.

The bladder is composed of the same number of coats as the ureters. It is lined with squamous cells.

THE URETHRA.

The urethra consists of two coats lined in different parts with different cells—in the prostatic portion with transitional cells; the middle portion with stratified columnar; and in the penile portion with columnar cells.

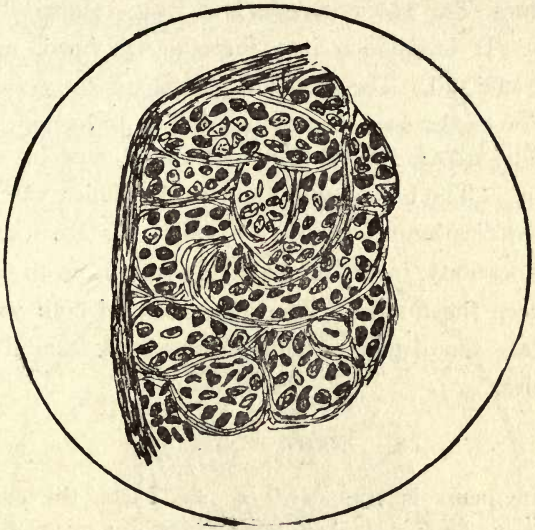
CHAPTER XV.

THE REPRODUCTIVE ORGANS.

The reproductive organs of the male are the testes, prostate glands, Cowper's gland and the penis.

THE TESTES.

The testes are composed of tubular glands, and produce the spermatozoa. They are covered with a capsule of fibrous tissue. The coverings of the testes are tunica vaginalis, tunica albuginea and tunica vasculosa. These coats dip down into the organ and divide it into lobes. The lobes are composed of convoluted tubules, straight tubes and a net work of tubes. The convoluted tubules are lined with four layers of epithelial cells, beginning on the basement membrane, and coming outwards. You see the parietal cells, then the mother cells, then the spermatoblasts and finally the spermatozoa—all these layers are found in the lumen of the convoluted tubule. And the spermatozoa are the



T. S. TESTICLE,

Showing capsule, septa of connective tissue and lobules.

generating cells. The straight tube and the network of tubes are a continuation of the same tube. These tubes all unite before they leave the epididymis and form the duct of the testes, called *vas deferens.* It consists of three coats, (a very thick muscular coat). The *vasa efferentia* makes up the epididymis. The globus major is the head of the epididymis and the globus minor is the tail of the same. The *vas deferens* constitutes the spermatic cord. The hard mass of fibrous tissue upon which rests the net work of tubules is called the medi-

astinum. The spermatozoön is a long, comma shaped body. It is divided into three parts: head, middle piece and tail. The *vesicula seminalis* is the expanded portion of the *vas deferens* in addition to having a few mucous glands of tubular type in its mucous membrane. "The blood supply enters the tunica vasculosa and mediastinum."—*Leroy*. Lymphatics are found in two locations: in the tunica albuginea and in the space between the tubules. The nerves are of both varities and are found in the spaces between the seminiferous tubules.

THE PENIS.

The penis is composed of two parts, the *corpora cavernosa* and the *corpus spongiosum*, having a large amount of connective tissue between them. The blood supply is derived from the *dorsal* and the *corpora cavernosa* arteries. The lymphatics empty into the deep lymphatics of the pelvis. The nerves are of both varieties. They are derived from the *pudic* and *hypogastric plexus* of the sympathetic. The nerves end in nerve corpuscles or end bulbs. The glands of the generative organs are of the tubular type.

THE OVARIES.

The ovaries are the female organs of generation, placed one on each side in the pelvic region. They are

covered with two capsules: the germ epithelial layer of cells, derived from the peritoneum and a tough band of fibrous tissue, called tunica albuginea. The substance of the ovary is divided into two parts, the cortical and medulla, which merge into each other. In the young ovary one of the cells of the germ epithelial layer becomes cut off and drops down into the medulla.

L. S. OVARY.—CAT.

1, germinal epithelium; 2, immature Graafian follicles; 3, stroma; 4, blood vessels in stroma; 5, a Graafian follicle from which the ovum has escaped; 6, 7, ova in different stages of development; 8, a fully developed ovum; 9, corpus luteum.

A band of fibrous tissue from the stroma encloses this cell. This band is called *theca folliculi,* and is divided in two parts, an inner and an outer. The first cell is called primordial ovum. Just inside of the theca folliculi is seen a granular zone, called membrana granulosa. Inside of this stratum is the discus proligerus. Now, inside of all this is the ovum, a large circular body, enclosed in two cell walls; viz: the *zona pellucida,* and the *vitelline membrane.* In this space between the ovum and the vitelline membrane is found the vitellus. Situated to one side of the *vitellus* is located the *germinal vesicle.* In the center of the *germinal vesical* is the *germinal spot.* A Graafian follicle that has lost its ovum is called a *corpus luteum.* The blood vessels enter the ovary at the attachment of the broad ligaments and pass into the theca folliculi. The lymphatics are most numerous in the medulla. The nerves are of both varieties, and supply the follicles.

THE FALLOPIAN TUBES.

The fallopian tubes, as all other tubes, consist of three coats. The mucous coat in these tubes is lined with ciliated epithelial cells. The blood vessels are the same as in the other cases.

THE UTERUS.

The uterus is the same in structure, only the muscular tissue predominates in this organ. The Nabothian

glands are in the mucous coat of the uterus. They are called the uterine glands.

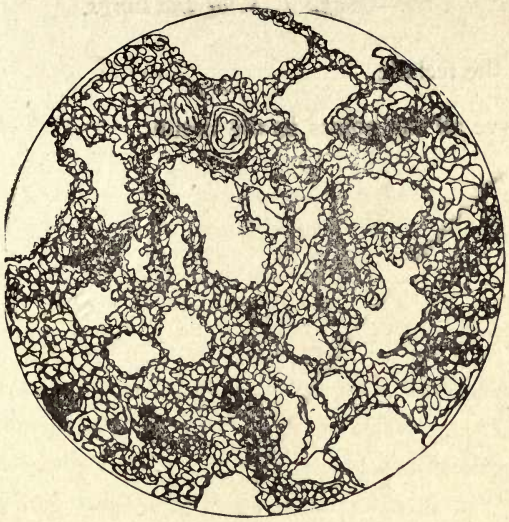
THE VAGINA.

The vagina is lined with stratified, pavement epithelial cells. The muscle tissue is arranged in two layers, an inner (circular) and outer (longitudinal), and is composed of the smooth variety. The region of the clitoris and the *labia minora* contains many sebaceous glands. In the region of the urethra, mucous glands are found called Bartholin's glands.

CHAPTER XVI.

THE RESPIRATORY SYSTEM.

This system comprises the nose, epiglottis, trachea, larynx, bronchi, lungs and pleura. These tissues are derived from the mesoderm and the epiderm. The nerve supply is from the cerebro spinal and sympathetic system. The epiglottis is chiefly yellow cartilage. Its mucous surface is covered sparingly with taste buds. The larynx is cartilagenous. The trachea is composed of three coats: mucous, submucous and fibrous. The mucous coat consists of ciliated epithelial cells, basement membrane and elastic fibers. The submucous coat consists of cartilage, glands, elastic tissue and non-striated muscle. The cartilage is of the hyaline variety and is arranged in the shape of a horseshoe. "The large bronchi are like the trachea in structure, only the cartilage is not arranged in a loop, but is arranged in plates."—*Sobotta.* A bronchus having a diameter not over 1 micron is called a bronchiole. They still have a layer of muscle and a layer of cubic epithelial cells. These bronchioles lead into the respiratory bronchioles,



LUNGS.

and these in turn lead into the alveolar ducts. The alveolus is divided into separate chambers, all communicating with one opening, called an infundibular space. The alveolus is lined with a single layer of epithelial cells. The blood vessels follow the course of the bronchus and break up into capillaries around the alveolus, and there the blood undergoes the gaseous change. The blood changes its name from venous to arterial. The lymphatics and nerves are scattered all in the substance of the lung tissue. The nerves are of both varieties, and they follow the course of the blood vessels.

Exercise No. 62.—Make T. S. of the lungs.

Note the technic.

Observe all the points in the tissue.

CHAPTER XVII.

THE SPECIAL SENSES.

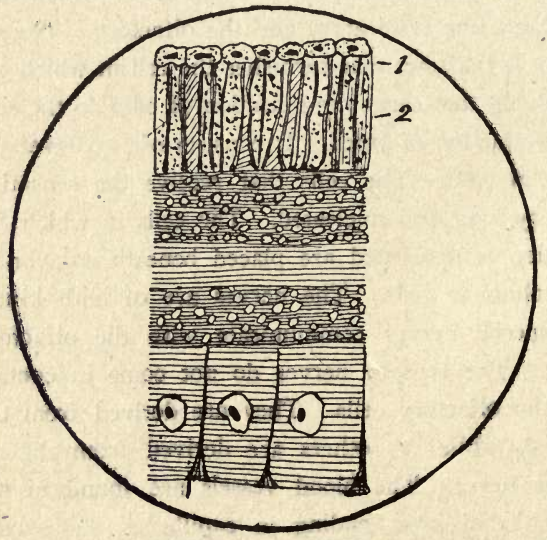
The special senses are located in the nose, eye, tongue, skin and ear.

THE NOSE.

The mucous membrane of the nose is divided into two tracts, the respiratory and the olfactory. The olfactory is the tract that furnishes the cell in which the nerves end that carry the sensation of odor to the center of olfaction or smell. It consists of two layers of epithelial cells. The cells that receive the sensation are very long and nucleated. The cells in which the sensation is generated are placed beneath a layer of tall, columnar cells. The nerves are of both kinds. The special nerves communicate with the olfactory center. The sensory nerves do not come in contact with the olfactory cells. They are derived from the trifacial, while the others are derived from the olfactory nerve. The blood vessels are found in the sub-epithelial layer ending in capillaries. The lymphatics are numerous.

THE EYE.

The eye is the organ of vision and consists of a hollow globe divided into two chambers: the anterior and posterior, separated by the iris and lens. In front of the anterior chamber is the cornea and behind is the lens. The posterior chamber is composed of the sclera, choroid and retina. The sclera is the white of the eye. The choroid is the coat between the retina and the sclera. The retina is the vision-producing membrane. The cornea is composed of five layers.



SECTION OF RETINA.

1, pigment layer; 2, layer of rods and cones.

The lens is a biconvex body, placed between the anterior and posterior chambers of the eye, and held in place by the suspensory ligaments. The anterior chamber is filled with a clear fluid called aqueous humor, and the posterior, with a fluid called vitrous humor. The lymphatics are found around the nerves of the eye and in a few other places. The nerves are derived from the ciliary nerves. The retina contains ten layers.

1. The layer called internal limiting membrane.
2. The layer of nerve fibers.
3. The layer of ganglion cells.
4. The inner molecular layer.
5. The nuclear layer.
6. The outer molecular layer.
7. The outer nuclear layer.
8. The external limiting membrane.
9. The layer of rods and cones.
10. The layer of pigment cells.

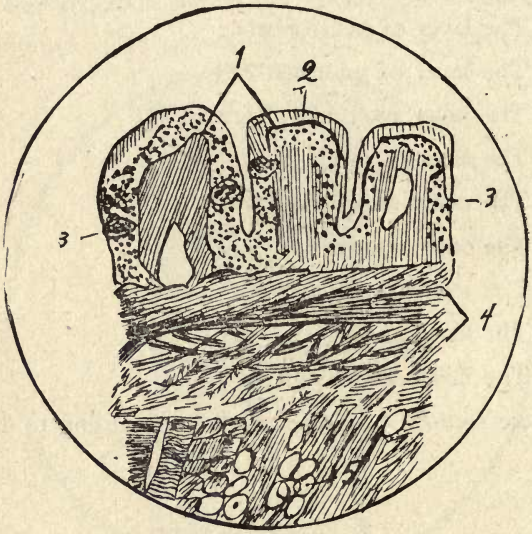
Make sections of tissue supplied, according to directions.

THE EAR.

The special organ of the sense of hearing is located in the ear. The ear is of mesodermic origin.

THE TONGUE.

The tongue is the special organ of the sense of taste, and is placed in the oral cavity. It is covered with a layer of stratified epithelial cells. The mucous membrane studded with the little projections called papillæ, upon which you find little taste buds. The muscle layers run in every direction. It is well supplied with blood vessels and lymphatics. The nerves are of



SECTION OF THE TONGUE.

1, mucosa, showing three papillæ, covered with squamous epithelial cells; 2, stratified epithelium; 3, taste buds; 4 muscular layer; 5, adipose tissue.

the sensory and taste variety. The papillae are of three varieties, filiform, fungiform and circumvalate. They all have a few taste buds connected with them. The tongue is divided into halves by the septum linguæ. In the tongue will be found several tissues of the body, viz: fat, muscle, epithelial cells and fibrous tissue.

Exercise No. 63.—Make T. S. of the tongue and examine.

PART II.
URINALYSIS

INTRODUCTION.

It always gives me pleasure to give my experience in our chosen profession, especially when it is in any way connected with the analysis of urine, a subject which I think is of vital importance in the diagnosis of diseases, since by it we may arrive at a definite conclusion as to the nature and location of the pathological state. The older physicians for many years depended entirely on a sugar and albumin test of urine. But that no longer satisfies the investigating mind of the scientific physician, since sugar and albumin are only two of the many pathological elements found in the urine. The methods of discovering the constituents are few and simple, and most of the methods require only a short time to complete them; so, you see, for time spent and money expended on one hand and knowledge gained and scientific treatment established on the other, there is no sort of comparison. Now, gentlemen, the value of these analyses will depend on our knowledge of pathology.

Pathology is only perverted Physiology. Since we know that the urine is the sewage of the body and that

it is the principal way by which the soluble material of the blood and tissues leave the economy, we naturally look to the urine to find any pathologic changes that might be taking place in the body. These changes are of two varieties: 1. An increase in the normal constituents of the urine. 2. An introduction into the urine of some foreign matter that may be acting as a poison; in fact, anything introduced into urine that is not normally found in it is a poison, illustrated in the case of poisons, as treated in Toxicology. This variety is of most interest to the specialist. The first variety is of most interest to the physician, since in ordinary diseases it is either an increase or decrease of the normal constituents of the urine. An analysis of the urine not only gives us an idea of the changes going on in the urinary tract, but in other places. A chemical examination is essential, since it gives an idea of the changes going on in the body a distance from the urinary tract, while the microscope only detects the solids in the specimen. How are these changes determined? We are now going to talk about the most important constituent of the urine, since by it the nitrogenous material leaves the body.

URINALYSIS.

The analysis of the urine is very important to the physician and surgeon. It consists of three departments, viz.: chemical, physical and microscopical. In order to gain any information from an analysis, it is necessary to understand the secretion of the urine. There are two theories as to the secretion of it, viz.: the capsule of Bowman, acting as a filter, separates the urine from the blood; the epithelial cells lining the uriniferous tubules secrete a part of the urine. In fact, both processes go on at the same time. The first is Ludwig's theory, and the second is Bowman's. The experiment of Heidenhain settled the question of the epithelial cells having a part in the secretion of the urine. He injected a coloring matter into the veins of an animal. After a suitable time he made sections of the kidneys of the animal, and found the coloring matter in the cells of the uriniferous tubules, but none in the capsule of Bowman. Other experiments prove the theory good, for when a patient has uremia, the uriniferous tubules have lost their epithelial lining, as shown by the microscope on analyzing the urine. The theory of Lurwig is proven by the change in the amount of

urine after drinking a large amount of water. The urine is increased in quantity but not in quality. (For the histology of the kidney, see the illustration of that organ.) The urine is the sewage of the system, and, therefore, contains the results of all the tissue metabolism. The changes the tissues undergo in the body you understand from the knowledge of physiology. The urine being a filtrate of the blood, it would be natural to suppose that it was of the same reaction as the blood; but not so. It is acid, while the blood is alkaline. The cause of this change was proven by C. H. Ralf to be the result of vital phenomena. The solutions necessary for an analysis of the urine are hydrochloric acid, sulphuric acid, acetic acid, nitric acid, liquor potassii, liquor sodii, liquor sodium aqua carbonate, liquor baric chloride, ammoniae and liquor magnesium sulphate, Hain's solution, silver nitrate solution, liquor plumbic acetate, alcohol, water and hypobromite solution.

The apporatus. A notebook, two dozen test-tubes and a test-tube rack, two conical glasses, spirit lamp, beakers and watch glasses. funnels, 2,000 c.c. vessel, graduates, filter paper, water bath, tripod, swab for cleaning the test tubes, a microscope and several pipettes. Other things will be added as we go on with the work.

The following solution should be made by the physician: hypobromite solution and Haines' solution. The others can be purchased at a drug store. The first is prepared as follows: 25 c.c. of water to 100 grams of sodium hydrate; the bromine and sodium hydrate must be kept in separate vessels. When ready to make the test, take 10 c.c. of the soda solution and 1 c.c. of the bromine, add equal amount of water, mix, then put the whole into the ureometer, then add the urine.

Technic.

1. Put the solution in the ureometer, filling the arm and one-half of the bulb.

2. Add 1 c.c. of urine to the solution; use a pipette.

3. Read the amount of *urea* in the specimen by means of the graduate on the arm of the instrument. This reading represents the amount in 24 hours in a liter of urine. The normal amount ranges from 20 to 33 grams. The *urea* is decreased or increased in many pathological conditions of the system. (For details on the subject, see Practice, and for source of urea, see Physiology; for composition and for reaction between the solution and the urea, see Medical Chemistry.)

In urinalysis all vessels must be clean. For chemical analysis, the specimen must be that of the urine representing the 24 hours urine, if not the whole.

For microscopic examination it is best to have the urine as fresh as possible. In the summer time it is best to add to each four ounces of urine 10 grains of sodium salicylate to prevent decomposition.

The physical properties of the urine are too well known to require special mention here. (See Physiology on the following points: color, odor, transparency, reaction and amount.) The color is straw; the reaction is acid; the odor is peculiar to itself; the amount ranges from 1000 c.c. to 1500 c.c.; normal urine is transparent. In disease all of this is changed, each change representing a pathological process. Sometimes several properties are changed in one case. (For diseases represented by the changes, see Practice.) The method of making the physical analysis is simple. Use the senses given you. For reaction use litmus paper. Specific gravity of the urine is determined by using the urinometer. The normal gravity is 1020 to 1025.

Method:

1. Fill the cylinder of the urinometer two-thirds.

2. Set it on a level table, and introduce the bulb of the instrument in the center of the cylinder; when the bulb comes to rest, read off the graduate on the stem of the bulb. In case you have not enough urine to find the gravity, you must add water enough to float the bulb; then multiply the gravity of the mixture by the

sum of the water and urine together. This is not practicable only in case the urine is of high gravity.

The specific gravity of the urine depends upon the solids in it. The most abundant solid is the *urea;* the other solids are in the shape of salts—therefore, the acids and metals are in the combined state. The tests for the metals are the same as the ones used in analytical chemistry; this is also true with the acids. The decomposition of urine is accompanied with a peculiar odor, caused by the *urea* breaking up into its constituents. The elimination of the *urea* represents the tissue metabolism, a process which is explained under the head of *Metabolism* in this text. The *urea* represents about two per cent. of the solids, and the solids represent about four per cent. of the urine. "Schroder's experiment is as follows: he injected into the liver by the portal vein a mixture of ammonium carbonate and blood, and on examining the blood in the hepatic vein, it was found to contain *urea* in abundance. This does not occur when the same experiment is performed with any other organ of the body, so that his experiment proves the great importance of the liver in urea formation."—*Kirk.*

The accumulation of urea in the body is called *uraemia,* and the same may be said of the accumulation of uric acid; only the term is uricacidaemia. There is

some discussion as to the relation between *urea* and *uric acid.* The ratio between them is as one to forty-five. (For further information see Medical Chemistry.) At certain times of the day the reaction of the urine differs widely. The time when it is acid is called the *acid tide,* and when it is alkaline it is called the *alkaline tide.*

What are the most abundant constituents of the urine?

What is said of the acids and metals in the urine?

What is the cause of the odor of stale urine?

Examine the spread of urine after the most improved method.

How do you make a microchemic-analysis?

UREA.

With the ureometer make a test for urea.

What is the normal per cent. of urea?

What does urea represent in the body?

Give Schroder's theory in reference to the formation of urea, as taught by an experiment.

Where is urea formed in the body?

What is urea accumulation in the blood called?

Is there any connection between urea and uric acid?

What is an accumulation of uric acid in the blood called?

How is uric acid formed for experimental purposes?

Take 10 c.c. of urine and 2 c.c. of hydrochloric acid; put the acid in the urine and set aside for two hours. Examine under the microscope.

Note the shape of the crystals.

What is meant by the acid tide and the alkaline tide?

MICROCHEMIC ANALYSIS.

Take a drop of urine to be examined and put it on the slide; also a drop of the reagent to be used. Place it on the slide near the urine, put a cover-glass on the drops, and watch the reaction.

Set a method by which you will always work in your own office or laboratory.

ABNORMAL CONSTITUENTS IN THE URINE.

Albumin in the urine is the first to consider. Albumin is coagulated by heat and certain chemicals, so we make use of these principles in our analysis.

Exercise No. 1.—Secure your key and clean out your locker. Keep your locker locked. Note the quantity of a liter as the demonstrator measures it out.

Each student should collect the urine for 24 hours from a patient and bring it to the laboratory to analyze.

Keep the urine in a tightly stoppered bottle to prevent evaporation. From the total quantity for the whole twenty-four hours samples should be taken to make tests.

SPECIFIC GRAVITY.

With the urinometer take the gravity of the urine and note the same. Dilute the urine one-half and take the gravity again. Note the result. Give the range of gravity in normal urine. Name what conditions will increase the gravity; also what conditions will decrease the same. (See Physiology.)

NORMAL COLOR.

What is the normal color of urine? Give the cause of the same. Note the color of ten specimens of urine. (Read Physiology on the urine.)

REACTION OF URINE.

The reaction of urine is due to what? Under what circumstances is it alkaline? How do you test the reaction of the urine?

COMPOSITION OF URINE.

Total grams	1,500.000
Water	1,440.000

Solids	60.000
Urea	35.000
Uric acid	.750
Sodium chloride	16.500
Phosphoric acid	3.500
Sulphuric acid	2.000
Ammonia	.625
Creatinin	.900
Chlorine	11.000
Potassium	2.500
Sodium	5.500
Calcium	.260
Magnesium	.210

TEST FOR CHLORIDES IN URINE.

Take 10 c.c. of urine, add to it 1 drop of potassium chromate; add to this silver nitrate until the color changes to red, noting the amount of silver added. Now calculate the amount of chlorine present. 1 c.c. of silver nitrate corresponds to .00586 grams of sodium chloride. A more practical method is to take a small amount of urine and add to it 1 drop of 1 in 8 silver nitrate solution. Note results. If a curdy precipitate occurs, which is not divided on shaking the urine, the

quantity is normal, or if it is divided easily, the quantity is diminished.

The presence of albumin in small quantity does not make a change in the reaction of the chemicals. (For clinical significance, see Tyson on Urine.)

PHOSPHATES IN URINE.

To 10 c.c. of urine add a small amount of ammonia. Warm gently until the earthy phosphates begin to separate. Set aside 10 or 15 minutes until they are completely settled; then measure the height of the precipitate, and if it is 1 mm. high, the quantity is normal. (For clinical see Tyson on Urine.) This is the test for the earthy phosphates.

THE ALKALINE PHOSPHATES

are determined by adding to the urine a small quantity of magnesium fluid, which precipitates the phosphates into a cloudy precipitate. This cloud is normal. The more the cloud, the more the phosphates.

SULPHATES.

To a small amount of urine add a drop of barium-chloride, and if a cloudiness is produced it indicates that a normal quantity of sulphates are present. If a

curdy precipitate is produced, it indicates an increased quantity. (For clinical, see Tyson on Urine.)

1. Make a standard solution of sodium phosphate (10.885 grains of well crystallized sodium phosphate) in distilled water and dilute to a liter. (50 c.c. then contains .01 centigram of phosphoric acid.

2. Make a saturated solution of potassium-ferrocyanide.

3. Make a solution of sodium acetate by dissolving 10 grams of sodium acetate in 100 c.c .of acetic acid c. p. and diluting the 1,00 c.c. with water.

4. Make a solution of uranium acetate, such that 1 c.c. will correspond to .005 milligrams of phosphoric acid made as follows: Dissolve the uranium acetate in water until the proper strength is obtained.

Step 1. Take 50 c.c. of standard solution of sodium phosphate in a beaker with 5 c.c of the solution of sodium acetate. Heat to 90 degrees C.

Step 2. Let the uranium solution run in until the warm mixture ceases to precipitate. This is done from a burette.

Step 3. Take a small amount of the potassium solution and place it on a clean white dish; then transfer a drop of the warm mixture to the dish containing the potassium solution. If the reddish brown precipitate

does not appear, continue the adding of the uranium solution until it does. Then read off the amount used. This is the amount that will precipitate 0.1 decigram of phosphoric acid. Now make a quantity of the uranium solution, say a liter.

SULPHATES.

Make a solution so that 1 c.c. of the solution of barium chloride will precipitate 12.50 milligrams of sulphuric acid prepared as follows:

1. Solution. Dissolve 30.50 grams of barium chloride in a liter of water.

2. Solution the same strength as the one above, but a different solution. Dissolve 27.57 grams of potassium sulphate in a liter of water.

CHLORIDES.

1. Make a saturated solution of potassium chromate.

2. Make a solution of silver nitrate such as 1 c.c. will precipitate 10 milligrams of NaCl, as follows: Dissolve 29 grams of stick silver nitrate in a liter of water.

ABNORMAL URINE.

Sugar solution. 30 grains of copper sulphate dissolved in one-half ounce of water; then add one-half

ounce of glycerine. Thoroughly mix. To this mixture add 5 ounces of liquor potassæ. Make 1 liter of this.

ALBUMIN.

Albumin is one of the abnormal constituents of the urine. The presence of the same is indicative of some lesion in the kidneys, especially in the uriniferous tubules, according to Bowman's theory. This may be a nephritis. The method of finding the presence of the same is simple. It depends upon the fact that albumin is coagulated under certain conditions. These we must now study. Heat the urine at the top for a short while and, if albumin is present, there will be a white ring at the junction of the cold and hot urine, which is the test for albumin.

Make an acid test for albumin. Take a small quantity of nitric acid, and place it in a test-tube and allow a small amount of urine to flow down the side of the tube. If albumin is present, there will be a white ring or zone at the point of contact. This is said to detect 1 part in 100,000 parts of urine. But this is not as delicate as the heat test.

GRAVIMETRIC METHODS.

Gravimetric methods are of extreme importance to the physician in cases of Bright's Disease. But the

method is so long that a busy physician has not time to follow it; so we must depend on some approximate methods, the best of which are to precipitate the albumin by means of boiling the urine and adding a few drops of nitric acid and setting it aside for twelve hours. Shake the specimen once or twice in order to get a uniform mixture of the materials in the urine. A more accurate way is to take an Esbach's albuminometer. (See your own drowings for the same.)

Method: Take the specimen of urine and put it into the apparatus to the graduation U, then put in the reagent to the letter R; put in the stopper; shake well; set aside until next period; read off the amount of albumin on the albuminometer.

Each graduation denotes 1 gram of dried albumin in a liter of the urine.

The next method is to use the centrifuge, which is very simple to use, and is very accurate in results. Learn to use the centrifuge.

The amount of albumin found in a specimen is very variable, but not over two per cent. in any case. This is a very large amount.

Sugar in the urine is the next important abnormal ingredient. The detection of the same depends on the power that sugar has of reducing the copper salts into cuprous hydrate, which is yellow or reddish yellow.

Hain's Solution is the best to use in the test. The mere change is not conclusive of the presence of sugar, since a reddish brown is the test for sugar—that is, the color desired, but we may judge from the color to a certain extent, whether sugar is present or not.

FERMENTATION TEST.

The fermentation test is performed as follows: Take a saccharometer and place in it a small part of a cake of yeast and fill it up to the bulb with the urine to be tested; set aside until the next period, and read off the amount of sugar in twelve hours in a liter of the urine. The graduations on one side of the apparatus represent grams to the liter; on the other, grains to the ounce.

PRACTICAL QUESTIONS IN NORMAL URINALYSIS.

Give the histology of a urine-bearing tube.

Give the theory of Ludwig in regard to the secretion of the urine.

Give C. H. Ralf's experiment on the cause of the change in reaction between the urine and the blood.

Give the histology of a glomerulus of the kidney.

Give the theory of Bowman in regards to the secretion of the urine.

Give Heidenhain's theory of secretion of urine as shown by an experiment.

Give the conclusions of the same experimenter.

How did you determine the amount of water in the urine?

How much urine voided in twenty-four hours?

How much urea in a sample of normal urine for twenty-four hours?

How did you make the urea test? Describe in detail the process.

What solution did you use?

What are the most abundant constituents of the urine?

Give the formula for urea.

Give the test for chlorides in urine, phosphates, lution.

Give the properties of urea.

Give the test for chlorides in urine, phosphates, urates, sulphates, sugar, albumin and bile.

What is the color, odor, reaction and specific gravity of normal urine?

Name and describe the apparatus used in determining the above.

Give cause of each property.

What is the consistence of normal urine?

Give five causes why the urine may be cloudy.

Give the test for nucleo-albumin.

Give the source of peptones in the urine; also test.

Give the test for bile in the urine.

Where is bile made in the body?

Give the indican test.

What is the presence of indican in the urine called?

MICROSCOPIC URINALYSIS.

Take a spread of the urine that you used when you precipitated the earthy phosphates and place it on the slide and examine.

Method: With a long pipette, take some of the urine from the bottom of the sedimentation glass and place it on the slide, cover with a cover-glass, using just enough urine to fill the space between the cover glass and the slide. Focus and make drawings of what you see. (See the Atlas.) Wipe off the slide, cover, and receive the spread that the assistant will give you; examine. (See Atlas.) Take a small quantity of urine and set aside for a while to settle and use the pipette on the same.

What method of making a spread is this?

Take the same amount of urine and do the same with it. Only use the centrifugal apparatus.

Why did you use the centrifuge?

Find the reaction of the urine you used in finding the earthy phosphates.

Find the specific gravity of the urine; note the difference between the normal gravity and this one.

What is the cause of the decrease in gravity?

Dilute the same urine to one-third and find gravity again.

Find the reaction of the urine you brought the first day, also the gravity.

What has taken place in the sample since you have been studying it?

In case you have not enough urine to find the gravity, how will you proceed to find it?

What is the gravity?

Test the sample of urine for urea after the hypobromite method.

What is the percentage of urea?

What ureometer do you use, and what advantage has it over other kinds?

What is the formula for urea?

Give the reaction between the solution and the urea.

EXAMINATION OF SEDIMENT.

Make a test of the sediment in the urine that has become cloudy from standing twenty-four hours, and determine the nature of the sentiment.

Method: Pour in the tube some of the urine and heat it, and if a cloudy precipitate appears, the sediment is due to earthy phosphates. If the cloud remains after the addition of an acid, it may be due to albumin; if it disappears, it may be due to the presence of phosphates; if it disappears on the addition of heat alone it is due to urates; if on the addition of an acid the precipitate disappears with the evolution of gas, the precipitate is due to carbonates.

What is the cause of ammoniacal odor in urine that has been standing for a while at a moderate temperature?

What causes the white precipitate that forms at the bottom of the vessel?

How can you tell when the alkalinity is due to a fixed alkali or to a volatile alkali?

Method: Test a sample of urine with litmus paper, then dry the paper, and if the blue remains, the alkalinity is due to a fixed alkali; if the blue disappears, it is due to a volatile alkali.

NUCLEO-ALBUMIN.

Test for nucleo-albumin. This is a very simple test. It is performed as follows: Take a small quantity of the urine to be tested and dilute the sample to reduce the salts that hold the albumin or mucin, which can

be differentiated by simply boiling it with a mineral acid. If a substance is produced that is reduced by cupric oxid the substance is mucin; if not reduced, it is nucleo-albumin.

PEPTONES.

Test for peptones in the urine: Place a drachm of Fehling's Solution in a test-tube, and put a small amount of urine in the tube, and if peptones are present a halo of a rose-colored hue will float, forming the test for peptones. (For the pathology for peptonura see Practice of Medicine.)

SUGAR TEST.

Moore's Test for sugar in the urine: Take a small quantity of urine in a test-tube and one-half as much strong alkali and boil, and if a reddish-brown or yellowish-brown precipitate appears, sugar is present. This precipitate intensifies as the boiling is continued.

HYDROBILIRUBIN.

Test for hydrobilirubin or urobilin: Add a small quantity of ammonia to the urine to be tested and filter the same. To the filtrate add a little chloride of zinc, and if a green color appears, urobilin is present.

UROERYTHRIN.

To a small amount of urine to be examined, add a small amount of neutral acetate of lead, and if a yellow color appears, uroerythrin is present. (See Tyson for clinical significance or the Practice of Medicine.)

URATES.

What is the brick-dust deposit in the urine?

The murexide test for urates is a practical test. Place a little urates in a tube and evaporate the same after adding a little nitric acid. A yellow residue is left. Add a little ammonia and the residue turns violet, which forms the test for the urates or uric acid. On the addition of potassium solution the color becomes blue.

Give the formula for ammonium carbonate, also ammonium carbamate. (See Medical Chemistry.)

BILE.

Test for bile in the urine: Take a small amount of urine and put it in a test-tube. Now let a small amount of commercial nitric acid run down the side of the tube on the urine. If bile coloring matter is present, a play of colors will be seen at the point of

contact of the urine and the acid (green, blue, violet, red and yellow). This is Gmelin's Test for Bile.—*Tyson.*

BLOOD.

This is a common occurrence. The presence of the same is detected by the color and the use of the microscope. The cause of the blood in the urine is a common question asked the physician. It is caused as follows: 1. Local or renal congestion. 2. Traumatism of the ureters, bladder, calculi, acute cystitis, and urethritis, simple and specific. 3. General diseases, such as malarial-fever. The color of the urine is the chief index to the beginning of the analysis. The microscope comes in next. It is the surest method to find the corpuscles in the urine before you say you are dealing with a case of *Haematuria.* Since there are so many other elements that resemble blood in color it is not safe to go by the color alone. There are many tests for blood, chemically, but these are not absolute, so we will not spend the time on them. (See Practice.)

PYURIA.

The presence of pus in the urine is called *pyuria.* There are two tests for pus in the urine. 1st. Any

strong alkali added to the urine will give a ropy precipitate. 2. Any good microscope will give you a fair outline of pus corpuscles, which is the best test for a busy physician.

What causes pus in the urine? Any lesion of the urinary tract, where pus is being formed, will result in a deposition of pus in the urinary tract. (These conditions are many.)

PART III.
BACTERIOLOGY

BACTERIOLOGY.

Bacteriology is the science of the minute organisms that inhabit the sea, land and atmosphere—in fact, every place that can be inhabited. Their morphology is simple. They are of three general classes, viz.: rods, spheres and curves.

Classify the spheres into cocci: Monococci, *i. e.*, one sphere; diplococci, two spheres; streptococci, spheres arranged in a chain; staphylococci, spheres arranged in a bunch. Staphylococci are divided into several names, according to the color of the growth on the medium.

GENERAL BIOLOGY OF BACTERIA.

There are six points to consider in the cultivation of bacteria: food supply, reaction, moisture, gaseous surroundings, temperature and light.

We will first consider the food supply, which is the medium on which they grow. It is, in nature, the different substances that they accidently fall on and grow; but, in the laboratory, it is the different *media* that we make for their sustenance or growth. Bac-

teria serve a useful purpose in nature by breaking up the complex substances that we find in nature into simpler ones.

The reaction of the medium is of importance, since many bacteria will not grow on any but an acid medium, and all but a very few require an alkalin medium.

Moisture is the next to consider. All bacteria require moisture; but some can live with less than others; some can stand drying for several days and then remain vital when placed in a suitable place for growth (for an example, take the anthrax).

The relation of bacteria to the gases of the atmosphere is another important consideration. Some can live without the oxygen, while others cannot live even a short while without it; still another variety can live with or without the presence of air.

Temperature is the next to be considered. The point where the bacteria will grow best is called the optimum temperature. This is usually the same as that of summer weather. The temperature in which they grow with the greatest difficulty is called the minimum temperature.

MEDIA.

1. Take beef extract.....3 grams
2. Peptone .....10 grams

3. Sodium chloride ..... 5 grams
4. Water ..... 1000 c.c.

Boil until ingredients are dissolved (fifteen to twenty minutes); add water from time to time to keep up the 1,000 c.c.; allow to cool slightly; measure out 1,000 c.c., and place them in a large flask.

Determine the Reaction.—Process: Take phenolphthalein as an indicator and normal 20 sodium hydroxide as an alkali; take 5 c.c. of the medium and 40 c.c. of distilled water; put 12 drops of the indicator into the mixture and proceed to add the alkali from the burette until the indicator shows sufficient quantity of the alkali, which can be observed by watching the color of the fluid as it changes from a colorless to a red. Calculate from this how much sodium hydroxide will be required to alkalinize the whole liter of medium; then add and boil forty-five minutes. Keep up the level by adding water; filter and distribute in the vessels you are going to use. Sterilize fifteen minutes each day for three successive days in the Arnold sterilizer.

PEPTONE SOLUTION.

Take sodium chloride, 0.5 grams; take peptone, 1.00 gram; water, 100 c.c.

We will make other media from time to time as they are needed. Everything is sterilized.

Agar and gelatine are simply bouillon hardened, or stiffened by them.

Everything must be labelled in the laboratory according to the model shown you.

Examine an inoculating needle, draw and learn the use of the same in more than one method.

Notice the demonstrator, as he inoculates one tube. Describe in full the process in your notebook.

Inoculate several agar and gelatin tubes, some on a slant surface and some on a square surface.

It is necessary that the glassware be thoroughly cleaned before it is used. Several methods have been suggested, but the best is the one which follows: First, take cleaning fluid and wash the vessels in it. The fluid is made as follows: dissolve 80 grams of potassium bichromate in 300 c.c. of warm water; when cooled, slowly mix the solution by constant stirring with 450 grams of sulphuric acid; store the liquid in a glass bottle with glass stopper.

CLEANING TEST-TUBES.

Wash them in warm soapsuds; then place them in the cleaning fluid twenty minutes; then pour the solution back into the bottle; wipe the tubes dry and drain

in the sink on the drain-board; wash them until the color of the solution disappears.

FLASKS AND PETRI DISHES.

Wash them with soap and water; then fill them with the fluid and allow it to remain ten minutes. Proceed as in the other case.

COVER GLASSES AND SLIDES.

Drop the cover glasses in the fluid one by one and let remain ten minutes. Take them out and wash them in alcohol; then place them in the sterilizer at a temperature of 160 degrees C for one hour. If a drop of bouillon is spread on a cover glass, the drop will spread out evenly if the cover-glass is clean.

PLUGGING TUBES.

This is a simple process, but much depends on the way you do it. The plug should be neatly made and firmly rolled just large enough to fit the test-tube or flask. They are usually made of cotton-batting, which should be sterilized before being used. The plug must not be too large or too small. It is best to have them just large enough to fit the tube or flask and no larger. The whole must be sterilized.

METHOD OF STERILIZING.

Place the vessels in the sterilizer and light the gas; watch the thermometer; keep the temperature up to 135 degrees C for one hour; then shut off the gas and let the temperature go down to 45 degrees C, and then open the door and take the vessels out. Store in the locker till wanted.

Light is another factor in the growth of bacteria. It is a very decided germicide to certain bacteria. It is probably the ultra violet and green rays that do the killing of the germs. But light and heat both are more certain to kill them than either heat or light alone.—*Muir and Ritchie.*

Movement of Bacteria.—This is variable. Some will move when they are in the act of reproducing. In what is commonly called the spore stage some move more rapidly than at other times; some less. Some substances will cause bacteria to move towards them, such as salts of potassium and sodium. This property is called *Chemiotaxsis,* positive and negative.

The Part Played by Bacteria in Nature.—As has been said, the function of bacteria is to break up the complex molecule into more simpler ones, as shown by the souring of milk, the curdling of cheese and the fermentation of sugar solution.

Saprophytic and Parasitic Bacteria.—The bacteria that grow on dead organic matter is called saprophytic, the variety that grow on living organic matter is called parasitic.

The variability of bacteria is of much importance, since the line of demarcation is not yet definitely determined.

The death of bacteria is usually determined by the fact that in a medium in which they were growing luxuriantly, on inoculating a new supply of the same medium they fail to grow.

Those agents which kill the greatest number of bacteria in the shortest time are carbolic acid and bichloride of mercury (the first in a solution of 1 to 1,000; the second in a solution of 1 to 20.)

These are called germicides. Those agents that so alter the medium that bacteria will not grow on it are called antiseptics.

The best germicides are heat, acids and the heavy metals in solution.—*Muir and Ritchie.*

CULTIVATION OF BACTERIA.

In order to study bacteria, it is necessary to have them growing apart from other bacteria. In nature this is not so since they are mixed up in general in the medium; it is only in the blood and tissues that we

find a few in the isolated state. When we have succeeded in obtaining a pure culture, we have only taken the germ from its mixed state and have it growing on a medium that is suited to its growth and development. These pure cultures are absolutely necessary to a successful study of the germ in question.

The Study of the Methods of Sterilization.—Heat is the best, whether in the form of dry or moist heat. This is the manner in which we do it: take a platinum wire and heat it to a bright red, then let it cool to a red heat; it is now ready to be inserted into the culture tube.

All instruments are sterilized in the bunsen before they are used. The glassware is sterilized by a dry heat. This is done by the use of the hot-air oven. Watch the demonstrator use the oven.

The method of using the oven is simple and will be used a great deal before we are through this work. This method is not used in sterilizing the media as it will evaporate the fluid media and scorch the solid before it will reach the temperature 135 degrees C.

Sterilizing by Moist Heat.—Boil the apparatus for five minutes in a covered vessel, and that will insure sterilizing for almost any variety of bacteria, but will not kill spores; so we boil the material about twenty minutes for three successive days. Steam sterilis-

ers are used in the following manner: in using the Arnold and the Koch Sterilisers, put the media in the steriliser at the same time you put in the water, and that will insure the media getting the same amount of heat that the water gets. Boil the whole fifteen minutes for three successive days. This is Tyndall's intermittent method. Each of these will be demonstrated in the class.

Sterilisation by steam at high pressure is a quick method but is not adapted to media work. It is carried on by means of the autoclave. Sterilisation by low pressure is adapted to one kind of media—that is, blood-serum, on Tyndall's principle.

Plating of Bacteria.—Take a sterilised platinum wire; let it cool slightly; then introduce it into the colony of bacteria and then into tube No. 1; shake well; then sterilise again; take a loop full from tube No. 1 and inoculate tube No. 2 with the same; sterilise; take a loop from tube No. 2 and inoculate tube No. 3; pour the contents into the same number of Petri dishes and number them according as the tubes were numbered; set aside until next day and note the change if any. This will be explained as we go along in our work. Always sterilise your needle before and after use. (The media must be melted.)

Hanging-Drop Culture.—This is made by simply taking the drop of media containing the growth and

placing it on the top side of a cover-slip and turning the cover-slip down on the slide and watching the motion or non-motion of the germ.

COLONY COUNTING.

1. Take a Petri dish and divide it off into equal segments and place it on the table, with a black surface under the dish.

2. Take 1 c.c. of the fluid to be used and carefully mix it with a tube of gelatine.

3. Pour the contents of the tube into the dish.

4. Incubate twenty-four hours at the room temperature.

5. With the aid of a lens count the colonies that appear in the dish. (Each colony represents a bacterium to begin with.)

BACTERIOLOGICAL EXAMINATION OF THE BLOOD.

1. Sterilise the part carefully with 1-1,000 perchloride of mercury; then wash off the mercury with alcohol; let the alcohol evaporate; wipe the part with a dry sterile cloth or absorbent cotton.

2. Prick the part with a needle prepared for the purpose by sterilising with dull heat.

3. Take a loop of the blood from the part and put it in the media, and if you get a growth, make the whole test for bacteria as directed further on.

Urine and water are examined in the same way (in general the vessels are cleaned).

Filtration of bacteria is an important process in the study of the organisms. It is done by means of a porcelain tube which is the only perfect filter known. Watch the demonstrator use the filter.

Examine water for Bacteria.—Take the water and place it in the plating dish and after a time count the colonies.

Method: Take a definite quantity of water and put it in a dish of agar-agar; put the same in an incubator for twenty-four hours; take out and count the colonies with the aid of a lens. Each colony represents a single germ.

Practical Use of the Analysis.—First, we find the B. Coli and B. Typhosus in water. The earth is another place where we find a great number of bacteria, *i. e.*, the B. tetani.

DETERMINING THE THERMAL DEATH POINT OF BACTERIA.

This is a simple procedure. Take some bouillon and inoculate it with some living bacteria; place it in a water bath and introduce a thermometer; turn on the gas and note the temperature; when the temperature reaches the body temperature take out one of

the tubes and inoculate a new tube with the contents of the hot tube; wait a while and take out another tube and proceed as in the above case, and so on, until you have taken out several tubes, all at a different temperature. Set the whole aside until the next period and note which one has the growth on it. It will usually be the one that stays in the bath the shortest time which will give the growth. The ones on which there is no growth are dead and the temperature at which they died is known by the reading on your tube, since you noted the temperature when you took out each eye of bouillon to inoculate the new tube.

DETERMINE THE STRENGTH OF ANTISEPTICS AND GERMICIDES.

Take some of the culture and subject it to the action of different strengths of the germicides in a Petri dish Any germ will serve for the test, or if the agent is a gas, we will place some pieces of blotting paper, which have been soaked in some good medium, and scatter them in a closed chamber and set the gas generator going. After a while take some of the paper and smear the surface of a sterile medium and the growth will not take place if the germicide is strong enough.

INOCULATING ANIMALS.

The mouse, guinea pig, rabbit, and white rat are the ones that are used most. Take the animal and sterilise

the parts to be inoculated; then take an eye of the growth on a needle after having made the incision at the root of the tail of the animal; inoculate the animal and keep him in a cage to prevent him from spreading the disease, if it is pathogenic. There are other methods. Fill a sterile hypodermic and inoculate the animal in the dorsal veins of the ear and observe the same precautions as in other cases. This determines the pathogenetic or non-pathogenetic properties of the bacteria.

Staining bacteria is a very important step in the study of bacteria. We will take that up when we come to stain the several kinds of bacteria.

Diagnosing bacteria is very difficult and is not free from error. But we do it this way: we observe the color or growth of the medium on which they grow best. Their action in the hanging-drop preparation, their morphology, their mode of bunching, their source, their relation to air, their chemical production, their action on an animal (pathogenetic property), their reaction with Gram's stain and analine dyes. These are the points by which we make a diagnosis of a colony of bacteria. A few others we will discuss as we come to them.

COVER-GLASS PREPARATIONS.

These are simple to make, but we must be careful not to contaminate the growth when we take the eye

from the tube to make the spread on the cover-glass. This is prevented by taking the needle and heating it to a bright red in the flame of the bunsen, and letting it cool a little before placing it in the culture or growth. The spreading of the material on the cover and drying in the air, and then passing it three times times through the flame, then applying the stain, according to the special process that we will learn as we go along, constitute the technic.

METHOD OF EXAMINING THE MATERIAL THAT A PHYSICIAN MAY BRING TO YOU TO EXAMINE.

Several cover preparations must be made; one to be stained with Gram's method, another to be stained with carbolfuchsin, one with gentian violet, and another with a mordant to stain the spores, if any are present.

Gelatin plates should be made and kept at room temperature. Now, if no growth has appeared on the plates in twenty-four hours, it is well to try some other medium. But, if a growth does occur, you must take a tube for your research that has only about 200 colonies on it. Now the first question to decide is, Are all the colonies of one or of many species? The question is answered by noting the characteristics of the different species. This is not always known. Note

all you can from the plate, and all you can from the hanging-drop culture, and all you can from the microscope and the reaction on certain media, also reaction on certain animals. The technic will be discussed in this chapter.

MICROSCOPIC EXAMINATION OF AN UNKNOWN.

Make the spread of the twenty-four hour culture; note the form, the size and the appearance of the protoplasmic contents regarding its reaction to a stain; the method of grouping.

Has it a capsule?

Do the bacteria stain with a watery stain?

Do they require a mordant to stain them or not?

How do they behave toward Gram's stain?

Are they motile?

Do they form spores?

Do they possess flagella?

What is the best temperature for their growth?

Growth on Media:—What are the characteristics of the growth? What is its relation to oxygen? What is the best temperature for its growth? Observe its growth on gelatine stab-culture, rate of growth, form

of growth, both on and in the substance, presence or absence of liquefaction, color, presence or absence of gas formation, odor and relation to reaction of media, streak culture, shake culture, plate culture and the condition of the colonies. Note the reaction on agar in the same way, also bouillon and special media. Note reaction on animals when inoculated.

Inoculating Animals.—The animal is either a rat, mouse, guinea-pig, or rabbit. Method: Take the animal, and sterilize the part selected for the operation, and inoculate the same and watch results. If the animal dies in twenty-eight days, the germ is pathogenic. But it usually dies in less time. The best location for the inoculation is in the dorsal veins of the ear or the root of the tail.

Autopsies on Dead Animals.—Method: Take a strong cord, a trough and the dead animal; tie the animal in the trough, soak the animal in carbolic acid solution to prevent the dry hairs from flying in the room during the autopsy, hence contaminating the air of the room; now open the body and examine. The animal should be burned after the operation.

INOCULATION AND INCUBATION.

To inoculate a tube of medium, take a culture and place it between the thumb and index-finger; take a tube of the medium that you are going to inoculate

and place it between the index and second finger; take an inoculating needle and sterilize it in the flame; let it cool a little; then place it in the culture; after having taken out the plugs as directed in the class room, insert the needle in the culture tube, then transfer an eye to the tube of medium and replug both tubes. Pass the culture on to the next. After having sterilized the needle, pass it also. Incubate the medium tube for twenty-four hours at room temperature after having labeled the tube as follows: name, date and medium.

Hanging-drop Preparation.—Take the hanging-drop slide and make a preparation in the usual way and examine the same. Note the following: shape, size, motility and spore-formation.

Dry Preparation.—Make a dry preparation after the usual manner, viz: Take the cover slip and clean the same by the use of alcohol; pass it three times through the flame; make a thin spread from the culture; dry in the air; pass over the flame until it is fixed, then place the whole under the misroscope and examine.

Watery-Stain Preparation.—Make a spread in the usual way and then apply the stain a few seconds; and wash in a glass of water; place under the microscope and examine.

Spore Stain.—Make a spread in the usual way and stain for spores.

Flagella Stain.—Make the spread in the usual way and stain for flagella.

Gram's Stain.—Place the spread on the cover in the usual way; apply the stain and note results.

This is what the reagents give us in regards to the diagnosis of bacteria. It is in order to take up the appearance of cultures of a studied germ. Inoculate all the media that you have and note the results on the same. Inoculate them in all the known ways; *i. e.*, stick, stab, smear and slant inoculations.

Note all the changes that will occur in twenty-four hours. This will help you very much in the diagnosis of the germ in hand.

Distribute the Media:—Take three tubes of agar-agar, and three tubes of gelatine and three of bouillon. Inoculate one bouillon and two gelatine, one slant and one stick in the same way as the gelatine. Incubate these for twenty-four hours. Note all the reactions that occur in that time.

QUIZ.

What are bacteria?

Give their morphology.

Where are they found?

What are the media on which they grow best?

How do we get rid of them?

What is the best means of getting rid of spores?

How hot must the medium be to kill all known spores?

What is the temperature to be obtained before you can take out the glassware when you are sterilizing it?

How do you use the hot-air sterilizer?

How do you lower the temperature in the hot-air sterilizer?

Are media sterilized in the hot-air sterilizer?

What are spores?

What are chromatic granules?

What do you learn from the use of the hanging-drop culture?

How did you make the cell for the culture?

Did you find any bacilli, any cocci, any spirilla?

How did you use the moist-sterilizer?

Why do you use the discontinuous method?

What is meant by sterilizing?

What is meant by pathogenic, saprophytic, chromogenic and pyogenic bacteria?

Are bacteria easily seen with the ordinary instrument?

At what temperature do they grow best?

Are all bacteria disease producers?

Are all bacteria chromogenic?

How did you fold the filter for filtration?

What is the advantage in this over the old way?

What medium did you make?

Are the principles the same?

Take a large tube of water and sterilize over the flame.

How can you tell when the tube is sterilized?

What is the temperature of boiling water?

Are the spores destroyed in the water—if not, why not?

If we use a steam boiler can we destroy the spores? If so, why can we?

What is the name of the steam boiler that we use in sterilizing cultures?

Name all the agents used in sterilizing, as a class.

Name two methods of using the dry heat.

What is the name of the rod and wire that you use in inoculating the medium in your hanging-drop culture?

How hot do you make the wire?

What kind of heat did you use in sterilizing the media?

What kind of heat do you use in sterilizing instruments?

Inoculation of Tubes.—Watch the demonstrator inoculate a tube of bouillon, then do the same. Incubate it for twenty-four hours.

Make a stained spread of the material given you and examine the same.

Determine the class to which the germs belong, whether cocci or bacilli.

Consult the atlas for the answer to the query.

Review the lesson you had the last period; note the likeness or unlikeness to this lesson.

Are all bacteria pathogenic or not?

Note their color in a growth?

Make a stained preparation of the germs at your table and examine.

Note in your notebook the points observed.

Determine the thermal death-point of streptococci in bouillon.

Method: Put some water in the water-bath; put the tube of culture in it, and heat to 56 degrees C.; let it remain for fifteen minutes.

Inoculate a second tube from this one, and store in your locker. Note the tube next day and see if there is any growth in the tube. If not, the thermal death-point is 56 degrees C. This method is the one we follow at all times.

Learn the theory of reproduction of bacteria. Note the color on the board which represents the stained germ and the stained spore.

FOOD SUPPLY.

The medium on which bacteria grows is termed food. We are to determine the best food on which they grow. The best ones are bouillon, agar-agar and gelatin. In order to determine the reactions of the medium after the germs have been growing on the medium, it must be of a composition the same as that of the tissues and juices of the body, or the same as that of the dead organic bodies. This we attempt to do when we make the different media. The properties of media which must be observed are clearness and solidity. The characteristic growth of the culture is observed on solid media.

Name the processes you went through when you made the stained preparation of the germs.

What is the best method to be followed when you make a preparation of bacteria—the stain or the other methods?

What are the three best stains for ordinary use?

You may use the oil-immersion lens when you make a good preparation.

Give the preparation of some of the stains in daily use.

Make a drawing to illustrate the morphology of bacteria.

Describe in detail the method of making a dry spread.

Watch the demonstrator make a plate of the air germs.

Note the date and medium.

Note the temperature of the room when the plate is made.

Give Tyndall's principle of intermittent sterilizing.

TYPHOID FEVER.

Typhoid fever is an infectious disease caused by the presence of the B. Typhosus. The B. was discovered

by Ebert and Koch, in 1880, and was first secured in pure culture from the spleen and affected lymphatic glands by Gaffky, in 1884.—*McFarland.*

The organism is a short, small bacterium, 1 to 20 microns in length and .5 to .8 microns in thickness.

The ends are rounded and sometimes they unite in chains, especially in the potato growth.

The organisms are actively motile, the motility being due to the presence of a long flagellum. They stain very well by Loffler's method, and they are used to demonstrate the presence of flagella in the study of bacteria in the laboratory. The organisms stain well with the ordinary stains. They lose their stain with Gram's stain.

The bacillus has no spore; the dark spot seen at the end of the germ is a chromatic granule.

The bacterium is both a parasite and a saprophyte. The bacterium is sometimes present in green vegetables which have been sprinkled with water contaminated with the organisms. This brings to mind a few years ago when the city ordered that certain springs be closed on account of the prevalence of the bacillus typhosus, and at the same time prevented the marketmen from sprinkling their vegetables with water from the brooks on their way to town. Their reason for such action is evident when we know that the organisms

thrive well in water. Their resistance to all germicides has been spoken of already. They grow well at room temperature, and boiling in water kills them, since they are killed at the temperature of 60 degrees C. This brings to mind another well-known fact, that the people in certain large cities are requested to boil all the drinking water before it is used. We know the reason why such a course is taken when we know the death-point of the germ. Cold has no effect on the germ, and certain chemicals must be very strong before they will kill them. The best chemicals are bichloride of mercury and carbolic acid. The bacilli are killed in a short time by drying. So a hot-air sterilizer will be all right for sterilizing the vessels used in the work on this kind of bacteria.

The best method of obtaining the typhoid germs is to take the animal after it is dead and get the germs from the spleen and lymphatic gland; but you can get them from the dejecta of the patient with difficulty.

The bacilli are sure to be present in the patient after the second or third week. Right here is the place to try Widal's Test.

The method of making a pure-culture from a patient's feces is as follows: Take several tubes of gelatin and melt the gelatin; have 10 c.c. of the medium in each tube; now add to each tube 1-10 c.c. of carbol-

ic acid. In the first tube put a loop full of the broken up *feces* and take a loop full from this tube and transfer to tube number two and so on. Next plate all the tubes and number them. The carbolic acid kills all the saprophytes and leaves only the bacillus typhosus and its near congener, the bacillus coli. The gelatin will not be liquefied.

For Widal's reaction read McFarland.

Serum diagnosis has been attempted. This is on the principle of attenuation of germs by the presence of another species.

The Bacterium Pyocyanus was the antitoxic growth used. This is on the principle of attenuation of germ by the presence of another species. This as yet has not come into use.

Make a spread of bacillus anthracis.

What is the morphology of the same?

Are they often found in man?

Give the approximate diameter of the germ and the length in microns.

Do you observe the morphology of the germs and spores in them,

Biology.—The bacilli grow well on all the ordinary media—best grown at 35 degrees C.; death-point, 60 degrees C. They withstand dry heat well, and

must be heated up to a very high degree before they are destroyed. Spores are of the usual variety. The action of the gastric juice kills them. They effect animals lower than man; therefore, they are a disease for the veterinary surgeon to study. The best method of getting rid of the dead animal is to burn it or bury it under a pile of slacked lime several inches deep.

Examine the tap water for bacteria by making the plate cultures in the usual way, as follows: Take the gelatin and melt it and pour it into the Petri dish; then take 1 c.c. of water from the hydrant and pour it into the dish on the medium, and note results in a few days.

Plate the pus in the vessel, and examine the same on the slide after staining.

Make a stained preparation of the urine on the table.

Tell to what class the bacteria belong, *i. e.*, if they are cocci or bacilli.

Some stain with blue and others with red.

Make a study of the bacillus of hog-plague.

Make the usual cover preparation; stain with fuchsin; make drawings of the same.

Examine bacillus prodigiosus; note the color of the colonies.

Use the colony counter and make calculation on the results.

Make a note of the method you would follow in making a bacteriologic diagnosis, stating the points that you would observe in each step.

Make a spread of bacillus tuberculosis and stain with fuchsin and methyline blue—the first, thirty minutes; the second, one-half minute. Examine under the oil lens.

Make a spread of spirillum cholera and stain with fuchsin.

Tell how you would determine the following points in the study of a certain germ.

First, the pathogenesis or non-pathogenesis.

Second, the chromogenesis.

Third, the motility.

Fourth, the morphology.

Fifth, the reaction to stains, and Gram's method.

Sixth, the reaction on gelatin.

Seventh, the reaction on all media.

Eighth, the source.

Ninth, the germicide for the germs.

Tenth, the thermal death-point for the germ.

Eleventh, the best temperature for its growth.

How do you prevent your mind from letting slip the points observed?

Make stained spread from the scrapings from your mouth; note the morphology.

Determine the death-point of streptococci as a class, after the following manner: Put a number of t. t. in an incubator and heat the water to 56 degrees C. and let remain fifteen minutes. Inoculate a tube of agar and place it in your locker until next period, and note if there is a growth or not on the medium. If there is, the germ or organism is not killed at the temperature of 56 degrees C.

Study Streptococcus and Sarcina. Note the effects on bouillon, and on agar-agar, on gelatin, and on potato. (See Sternberg on Streptococcus.)

See McFarland on biology of bacteria and note the points of interest to you as a bacteriologist. See what he says about the appearance of the colonies on the different media; note the similarity between his description and your notes. Note the appearance of the colonies on a gelatin plate.

Note the color of the growth of bacillus prodigiosus, sarcina luteus, staphylococcus-pyogenes.

Make a plate of gelatin and inoculate it with a drop of water to see if there are any bacteria in the water.

Study spore formation of bacteria.

Study suppurative diseases, their causes and cure.

Study the cause of gonorrhoea; the method of making the spread for the discovery of the cocci.

Study mixed infection.

Make a cover spread of the pus on the table and classify the germs in it.

INFLUENZA.

The disease Influenza is a widespread disease and is more prevalent in winter than in any other time. It is caused by the presence of a certain bacillus called Bacillus Influenzae. They are short rods 1.5 microns in length and .3 microns in breadth. The bacilli sometimes occur in chains and sometimes in bunches. They lose the stain by Gram's method. They are non-motile and do not form spores. They are best stained with carbol fuchsin. They grow best on blood agar and can be grown best on the agar by smearing the medium with the sputum of the patient. The lower animals' blood is as good as that of the human subject. The bacilli grow well at room temperature; their optimum temperature is that of the body. The power of resistance of this organism is of a low order. The mode of distribution of this bacillus is by direct contact with the mucus and pus of the person affected with influenza. The location in the body is unusally

in the respiratory organs. They are always mixed with other organisms. The purest cultures can be secured from the greenish yellow-pus that may be collected from the person so affected. As the disease advances, we may be able to find the germs in the leucocytes of the blood. It is a peculiar fact that the bacilli remain in the sputum for a while after the disease is cured.

Method of Making the Examination.—Take some of the greenish yellow pus and make the usual cover-glass preparation, and stain the same with carbol-fuchsin.

Give the use of the agglutinating test of Widal.

What is the thermal death-point of bacillus typhosus?

Watch the demonstrator use the anaerobic culture tube.

Widal's Test is as follows: Take one drop of the blood of the patient and mix it with nine drops of water; place one of these drops on a cover-glass; make ready a hanging-drop slide; now transfer one drop of a culture of bacillus typhosus and examine at once. If an agglutination occurs within fifteen minutes the patient has typhoid fever.

Make a spread of bacillus anthracis and examine it, using the oil lens. Study spore-formation from this bacillus.

What is the theory of immunity?

Express your opinion on the subject.

Give the theory of phagocytosis.

Demonstrate the anaerobic culture tube.

Experiments on Animals.—These are for the purpose of demonstrating the pathogenesis of the germ. Take the animal and put it in Voge's Holder, head downward. Take an antiseptic solution, sterilize the part selected for the injection, and at the same time sterilize your syringe in hot water. Now remove the lid of your Petri dish and draw the required amount of growth in the syringe. (The syringe is graduated in c.c. and 1-10 of c.c., so that you can get a definite amount.) Inject the material in the animal and put the animal in the cage until next period. Examine the animal and see if it shows signs of disease.

Taking the Temperature of an Animal.—Take the animal and place it in the Vogue, head downward; put the thermometer in the rectum, and note degree.

Make a spread of the material on the table and stain with fuchsin.

Bacillus Pyocyaneus is a short rod. It is three microns in length and has one flagellum; does not form spores; frequently forms chains of four or six; can exist with or without oxygen. It stains well with

the ordinary stains and will not retain Gram's stain. It is the cause of green pus in a wound. It produces the green color to the pus of old sores.

Stain a spread of bacillus Flouresence Liq., and note the likeness of the two.

Stain the dejecta at your desk and make out the morphology of the species, and try to differentiate the colon bacilli from the typhoid bacilli, if such are present. Consult the atlas and find the germs which are most like the ones on the slide.

What lesson of practical value can you get from this experiment?

How do you determine the power of any germicide? Note the process in your notebook.

How would you get the material to the city bacteriologists?

Name five diseases which you must report to the city authorities.

How would you make an examination of milk in case you were called on to do so?

What bacteria are most likely to be in the milk?

TECHNIC, REAGENTS AND STAINS

From OSBURN'S MANUAL.

TECHNIC, REAGENTS AND STAINS.

VEGETABLE SECTION.

NO. I. METHOD FOR STAINING FRESH VEGETABLE SECTIONS.

(1) Apply section to slide and add rosanilin violet, one to five minutes.

(2) Wash in water to remove excess of stain.

(3) Dry with blotting paper and add glycerine to dehydrate.

(4) Remove excess of glycerine and add glycerine again to thoroughly dehydrate.

(5) Wipe off excess of glycerine and add xylol twice.

(6) Apply to cover-glass a drop or two of xylol-balsam, and, having wiped off the excess of xylol from the slide, drop it gently (balsam down) upon the section. Then apply general pressure with dissecting needle to spread out the balsam.

(7) Label and keep in a horizontal position until the balsam is hardened.

Vegetable Sections.—To stain paraffin or celloidin sections of plant structures, the methods are practically the same as those given below for animal sections.

ANIMAL SECTIONS.

NO. 2. CARMINE METHOD FOR FREE SECTIONS.

(1) Apply section to slide and wash with thirty-five per cent alcohol.

(2) Add lithium carmine sufficient to cover section, one to five minutes.

(3) If necessary, remove excess of stain with acid alcohol, five to ten seconds.

(4) Dehydrate with increasing strength of alcohol —thirty-five per cent, seventy-five per cent, and ninety-five per cent, and absolute.

(5) Wipe off excess of alcohol, and when section is partly dry add creosote to clear up, five to ten minutes.

(6) Wipe off excess of creosote and mount with balsam.

(7) Centre cover-glass and apply pressure to spread out the balsam.

(8) Label and lay aside in horizontal position, cover-glass up, until the balsam hardens.

NO. 3. CARMINE METHOD WITH AFFIXED PARAFFIN SECTIONS.

(1) Apply to the center of the slide a thin layer of collodion-clove-oil mixture.

(2) Center and attach the section, applying the heat of a spirit or Bunsen flame.

(3) Immerse in xylol two minutes and in turpentine ten minutes to remove paraffin. Sections immersed in turpentine alone should remain twenty minutes.

(4) Wash with alcohol, decreasing strengths, using thirty-five per cent alcohol last.

(5) Apply lithium carmine, one to ten minutes.

(6) Remove excess of stain with acid alcohol.

(7) Dehydrate with alcohol, increasing strengths.

(8) Dry and clear up with creosote, five to ten minutes.

(9) Wipe of excess of creosote and mount in balsam.

(10) Center cover-glass.

(11) Label and lay aside in horizontal position until balsam hardens.

NO. 4. CARMINE METHOD FOR AFFIXED CELLOIDIN SECTIONS.

(1) Center section and affix with collodion mixture.

(2) Stain with lithium carmine, one to five minutes.

(3) Remove excess of stain with acid alcohol, five or ten seconds.

(4) Apply seventy per cent alcohol.

(5) Apply eighty per cent alcohol.

(6) Apply ninety-five per cent alcohol a few seconds.

(7) Clear up with creosote.

(8) Remove excess of creosote with blotting paper.

(9) Mount with balsam and center cover-glass.

(10) Label and lay aside in a horizontal position.

NO. 5. HAEMATOXYLIN METHOD FOR FREE SECTIONS.

(1) With section on slide, apply alcohol of the same strength as staining solution.

(2) Stain with diluted hæmatoxylin, one to ten minutes.

(3) Remove excess of stain with thirty-five per cent alcohol.

(4) Dehydrate with alcohols, increasing strengths.

(5) Clear up with creosote or cedar oil.

(6) Center section and apply balsam and cover-glass.

(7) Center cover-glass.

(8) Label and lay aside in horizontal position until balsam hardens.

NO. 6. HÆMATOXYLIN METHOD FOR AFFIXED SECTIONS.

(1) Apply to slide a thin layer of egg-albumen and glycerine.

(2) Center section and flatten it by gently touching with end of finger.

(3) Apply heat of flame until paraffin melts (sections that have been flattened upon water should be heated much longer than others).

(4) Remove paraffin with xylol or turpentine.

(5) Remove xylol, etc., with alcohol, decreasing strengths.

(6) Stain with diluted hæmatoxylin, one to ten minutes.

(7) Remove excess of stain with thirty-five per cent alcohol.

(8) Dehydrate with alcohol.

(9) Clear up with creosote, five to ten minutes.

(10) Wipe off excess of creosote and mount with balsam.

(11) Center cover-glass.

(12) Label and keep in horizontal position until balsam hardens.

NO. 7. HAEMATOXYLIN METHOD FOR AFFIXED CELLODIN SECTIONS.

(1) Center section and affix with collodion mixture.

(2) Stain with diluted hæmatoxylin, one to ten minutes.

(3) Remove excess of stain with acid alcohol.

(4) Apply seventy per cent alcohol.

(5) Apply eighty per cent alcohol.

(6) Apply ninety-five per cent alcohol a few seconds.

(7) Clear up with creosote, five to ten minutes.

(8) Remove excess of creosote with blotting paper.

(9) Mount with balsam and center cover-glass.

(10) Label and lay aside in a horizontal position until balsam is hardened.

NO. 8. HAEMATOXYLIN-EOSIN METHOD.

(1) If desired, affix section to slide with egg-albumen and glycerine or collodion and clove-oil.

(2) If a paraffin section, remove paraffin with xylol or turpentine or both; remove xylol, etc., with alcohol.

(3) Apply thirty-five per cent alcohol.

(4) Stain with diluted hæmatoxylin, one to five minutes.

(5) Apply thirty-five per cent alcohol to remove excess of stain.

(6) Stain with alcoholic eosin about five minutes.

(7) Apply ninety-five per cent alcohol to remove excess of stain and dehydrate.

(8) Clear up with cresote.

(9) Remove excess of creosote and mount with balsam.

(10) Center cover-glass and label.

(11) Lay aside in horizontal position until balsam hardens.

Staining Unicellular Organisms.

It is often desirable to examine materials without staining. This is accomplished by placing upon the

glass slip a drop of the material to be examined and applying cover-glass. A hair placed under the cover glass will prevent the object from being crushed and allow of free motion in the case of living organisms. Should it be desired to stain such preparations, two methods may be pursued, irrigation and cover-glass staining.

NO. 9. IRRIGATION AND STAINING MICRO-ORGANISMS.

(1) Place upon the slide a drop of material to be studied.

(2) Apply cover-glass.

(3) At the edge of the cover-glass, by means of a pipette, place a drop or two of the reagents or stain.

(4) By means of a triangular piece of blotting paper applied at the opposite edge of the cover-glass, absorb the moisture from the preparation, thus drawing under the stain.

NO. 10. COVER-GLASS PREPARATIONS.

(1) Make a thin spread of the substance to be examined upon a sterilized cover-glass.

(2) Using a Cornet forceps, dry the preparation by holding it between the fingers above the flame.

(3) When dry pass the cover-glass three times through a flame, keeping the preparation up.

(4) Apply stain.

(5) Wash in distilled water by dipping the cover-glass in the water two or three times.

(6) Examine as a water mount or, if desired, dry and mount in balsam.

(7) Label and lay aside in a horizontal position until balsam hardens.

Note.—The above method may be used for all simple staining. Special methods, however, are often used and they will be given as required.

LABORATORY EXERCISE.—*Centering and labeling.* Upon the under side of your box-cover make an outline of a slide. The pencil should have a needle point. Connect opposite angles and place over the intersection of the lines a cover-glass. Be sure that the centre of the cover-glass coincides with the center of the diagram. Now, carefully make an outline of the cover-glass. This outline may be used for centering both the sections and the cover-glass. Make a drawing of this outline and also a drawing of a slide with labels and cover-glass *in situ.* Fill in the forms of labels in second diagram, using the following data: A transverse section of the muscle of a normal

cat was stained with lithium carmine and mounted in balsam in October 1, 1891, by John Smith.

Drawings. For this work the student should provide himself with a No. 5 or a No. 6 H Faber pencil, a small rule or triangle and a sheet of thin blotting paper. The pencil should be kept sharpened to a needle point. The majority of students will say: I cannot draw. An honest and faithful effort will often produce gratifying results. Let every line mean something. Be scrupulously neat in all your work. Remember that this work will furnish a better exhibit of character and ability than any other task of the laboratory.

Abbreviations. The following abbreviations are employed in this text:

Transverse section—T. S.
Longitudinal section—L. S.
Vertical section—V. S.
Low power—L. P.
High power—H. P.
Cubic centimeter—c. c.
Micro-millimeter—*u*.
Millimeter—mm.
Gram—g.

REAGENTS AND STAINS.

In preparing the following regeants it is well to remember that the weight of a cubic centimeter of water is one gram, and that a liter contains 1,000 cubic centimeters. The formulæ that are given are those most commonly used and are briefly stated:

NORMAL FLUIDS.

Distilled water.—A supply of distilled water should be constantly at hand for the preparation of the reagents and stains.

Normal saline.—This is prepared by dissolving one part, by weight, of sodium chloride in 150 parts of distilled water.

MACERATING FLUIDS.

Dilute alcohol.—This may be prepared by mixing one part of ninety-five per cent alcohol with two parts of distilled water. Other fluids used for this purpose are solutions of potassium bi-chromate, two per cent, and caustic potash, twenty-five per cent.

DECALCIFYING FLUIDS.

Picric acid.—Make a saturated aqueous solution of picric acid. This is an excellent fluid for decalcifying

bones, serving at the same time as a staining reagent. Crystals should be added from time to time, so that some undissolved crystals will always remain in the bottom of the vessels.

Nitric acid.—Use a ten per cent volumetric solution in water. Decalcification occurs in five to ten days.

FIXING REAGENTS.

Absolute alcohol.—Specimens should remain in this reagent from one to six hours, according to size.

Perenyi's fluid—

Nitric acid (ten per cent).....40 cc.
Chromic acid (0.5 per cent).....30 cc.
Alcohol .....30 cc.

A good reagent for embryos and adult tissues. Time, three to twelve hours; dehydrate with alcohol.

Erlicki's fluid—

Potassium bi-chromate .....2.5 grams.
Cupric sulphate .....0.5 grams.
Water .....100 cc.

Aqueous Solution—

Corrosive Sublimate—

Corrosive sublimate .....1 gram.
Water ..... 95 cc.

Alcoholic Solution—

Corrosive sublimate .....1 gram.
Alcohol (95 per cent).....99 cc.

Used for special purposes and specially for alimentary tract. Time, twenty-four hours, hardening in alcohol, to which a few crystals of iodine have been added.

Chromic acid.—Use a 0.5 per cent solution, dehydrating with alcohol in the dark.

Muller's fluid—

Potassium bi-chromate ....25 grams.
Sodium sulphate .....10 grams.
Water ..... 1,000 cc.

Pulverize the solids before adding water, and use a piece of camphor in the solution to prevent the formation of fungi. Good for general use and especially valuable for central nervous system. Requires from two to six weeks. Wash in water for several days and dehydrate with alcohol.

Flemming's fluid—

Chromic acid (one-per cent solution).
....46 cc.

Osmic acid (two per cent solution).....
....12 cc.
Glacial acetic acid.....3 cc.

Especially valuable for delicate tissues. Time, twenty-four hours; dehydrate with alcohol.

HARDENING REAGENTS.

Muller's fluid, corrosive sublimate solution, chromic acid, and others of the reagents named above may be used for hardening purposes. For general use, *alcohol* will be found invaluable. The tissue should be passed through increasing strengths of alcohol, seventy per cent, eighty per cent, ninety per cent ninety-five per cent, and absolute. It should be allowed to remain twenty-four hours in each, except that one to six hours will suffice for absolute alcohol. Ethyl alcohol should be used, or, in lieu of this, methyl alcohol makes a good substitute. To prepare absolute alcohol, dehydrated copper sulphate may be added to ethyl or methyl alcohol. This will absorb the water present.

EMBEDDING MEDIA.

Paraffin and celloidin are extensively used for embedding tissue.

FIXATIVES.

Collodion and clove oil mixture.—Mix one part of collodion with three parts clove-oil.

Egg-albumen and glycerine.—Filter the whites of several eggs and add to the filtrate an equal volume of glycerine. To the mixture add a few drops of carbolic acid or a small piece of thymol to prevent putrefaction.

PARAFFIN SOLVENTS.

Xylol, turpentine, chloroform, and benzole are commonly used to remove paraffin from sections. A good plan is to immerse the slide containing the section for a few moments in xylol, and then transfer to turpentine for ten minutes.

STAINING SOLUTIONS.

The following staining preparations are those most frequently used, and will be found adequate to the work required in this text. Should others be needed, the formulæ can be obtained from more advanced works.

Hanstein's rosanilin violet—

Methyl violet.....1 gram.
Fuchsin .....1 gram.
Distilled water.....100 cc.

Note.—This is a valuable stain for vegetable sections. It should be diluted for use as desired.

Lithium carmine—

Carmine .....2.5 grams.
Lithium carbonate (saturated solution).....100 cc.

The carmine should be dissolved in cold solution. Sections stain rapidly, and should be decolorized with acid alcohol.

Daldfield's haematoxylin—

1. Hæmatoxylin ..... 1 gram.
2. Absolute alcohol.....6 cc.
3. Amonium alum (saturated sol.) ..... 6 cc.
4. Glycerine .....25 cc.

Methyl alcohol .....25 cc.

Process.—Dissolve (1) in (2); add this solution to (3); expose to air and light for a week; filter and add (4) and (5); allow it to stand for a long time exposed to air and light.

Alcoholic Eosin for Sections.—Make a saturated alcoholic solution. This is used as a ground stain in connection with hæmatoxylin; also as a blood stain.

Magenta for blood, etc.—

Magenta	1 gram
Alcohol (eighty-five per cent)	50 cc.
Water	150 cc.
Glycerine	200 cc.

Meythelene blue for blood—

Make a saturated aqueous solution.

Carter's carmine mass for injecting—

Carmine	3 grams
Strong ammonia	6 cc.
Glacial acetic acid	6 cc.
Coignet's French gelatin	7 grams
Water	80 cc.

Process.—"Place the finely cut gelatine in 50 cc. of water for five hours; dissolve the carmine in mortar with a little water and add the ammonia; let stand for two hours and then pour into a bottle, rinsing the mortar with the remainder of water; place the gelatin and water on a water-bath until the gelatin melts. To the carmine fluid add the acetic acid, a few drops at a time (rinsing mortar thoroughly) until the fluid becomes crimson. To the melted gelatin add the crim-

son carmine, little by little, with continual stirring. Keep in a cool place with surface covered with methylated spirit. When wanted for use, dissolve on water-bath and filter through flannel wrung out of hot water." (Fearnley's Method.)

CLEARING AGENTS.

Those commonly used are cedar oil, creosote, clove-oil, xylol, and aniline oil. Clove oil cannot be used with celloidin sections.

MOUNTING MEDIA.

Glycerine jelly and Canada balsam are commonly used for mounting purposes. For the laboratory, balsam will be found a satisfactory medium. Should xylol be used for clearing, the balsam should be dissolved in xylol. Chloroform balsam may be used in in xylol. Chloroform balsam may be used in sections

For the formulæ of reagents and stains required for work in bacteriology and urinalysis, the reader is referred to the chapters in which is discussed the micro-technique of these subjects.

MICROSCOPIC TECHNIQUE.

I. REAGENTS AND STAINS.

(1) DECOLORIZING SOLUTIONS.

Twenty-five per cent aqueous solutions of hydrochloric, nitric, and sulphuric acids may be used for decolorizing.

(2) ACID ALCOHOL.

Hydrochloric acid	1 part
Alcohol (seventy per cent)....	100 parts

(3) IODINE SOLUTION.

Iodine	1 gram
Potassium iodide	2 grams
Water	90 cc.

(4) CARBOL FUCHSIN.

Fuchsin	1 cc.
Alcohol	10 cc.

Dissolve and add 100 c.c. of five per cent solution of carbolic acid. Filter.

(5) ACID METHYLENE BLUE.

Sulphuric acid.....16 cc.
Water .....90 cc.
Methylene blue.....2 grams

This stain should be prepared fresh from time to time. The carbol fuchsin improves with age.

(6) LOFFLER'S ALKALINE METHYLENE BLUE.

Concentrated alcoholic solution of methylene blue .....30 c.
Potassium hydrate (aqueous solution 1-10000) .....100 cc.

This is especially useful in staining the bacillus of diptheria.

(7) ANILINE-WATER GENTIAN VIOLET.

Aniline oil .....5 cc.
Water .....100 cc.

Mix, shake vigorously, filter; the fluid after filtration should be perfectly clear; add

Alcohol .....10 cc.
Alcoholic solution of gentian violet.11 cc.

This solution should be freshly prepared about every two weeks.

(8) LOFFLER'S MORDANT FOR FLAGELLA.

Tannic acid.....2 grams.
Water .....8 cc.
Saturated solution of ferrous sulphate .....5 cc.
Saturated alcoholic solution of fuchsin .....1 cc.

(9) ANILINE-WATER DYE FOR STAINING SPORES.

Saturated alcoholic solution of fuchsin or gentian violet.... 11 parts
Aniline oil water .....100 parts
Abs. alcohol ..... 10 parts

Keeps well for ten days.

(10) AQUEOUS STAINS.

Saturated aqueous solutions of fuchsin, gentian violet, and methyline blue will be found useful for all simple staining.

(11) ALCOHOLIC SOLUTIONS.

Saturated alcoholic solutions of fuchsin, gentian-violet, and methyline blue should be kept on hand to

be used in simple staining and in connection with other stains.

II. STAINING METHODS.

(1) SIMPLE STAINING.

This consists in using a single stain.

(2) DOUBLE STAINING.

This consists in using two stains, one to stain spores, protoplasm, etc., and the other as a ground stain. The following methods will illustrate double staining:

STAINING OF SPORES.

(a) Make a cover-glass spread, dry and pass three times through the flame.

(b) Add aniline-water gentian-violet.

(c) Heat until the preparation begins to boil; remove for a minute. Repeat this process six times.

(d) Wash in three per cent hydrochloric acid-alcohol one minute.

(e) Wash in water.

(f) Counter-stain with aqueous methylene blue half a minute.

(g) Wash in water.

(h) Dry and clear up with xylol.

(i) Mount in balsam.

STAINING OF FLAGELLA.

(a) Mix upon the cover-glass a portion of the culture with a drop of water, using care not to break off the delicate flagella.

(b) Dry and pass three times through a flame.

(c) Apply Löffler's mordant one minute, warming gently.

(d) Wash in water.

(e) Stain with aniline-water fuchsin.

(f) Wash in water.

(g) Dry and mount in balsam.

GRAM'S METHOD FOR BACTERIA.

(a) Make a cover-glass preparation by the usual method.

(b) Stain with aniline-water gentian-violet solution, two to five minutes, warming slightly.

(c) Add Gram's iodine solution one and one-half minutes.

(d) Apply alcohol, repeatedly, long as stain continues to come away from the preparation.

(e) Wash in water and examine as a water mount.

(f) If desired, dry and mount in balsam.

GABBETT'S METHOD FOR TUBERCULOSIS, ETC.

(a) Make a cover-glass smear of the sputum, pus, blood, or urine to be examined. After the preparation is dry, affix by passing three times through the flame.

(b) Using a Cornet forceps, apply carbol-fuchsin five to ten minutes, heating until steam appears.

(c) Wash in water.

(d) Apply acid methylene blue for one minute.

(e) Wash in water.

(f) Dry and mount in balsam.

Staining Tissues for Bacteria.

Tissues may be stained in Gram's method by the following process:

METHOD FOR STAINING BACTERIA IN SECTIONS.

IN SECTIONS.

(a) Using an aqueous solution of fuchsin, gentian-violet or methylene blue; apply stain for about five minutes.

(b) Wash in water.

(c) Apply an aqueous solution of acetic acid, one per cent, for one minute.

(d) Apply alcohol two minutes.

(e) Clear up with xylol.

(f) Mount with balsam.

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